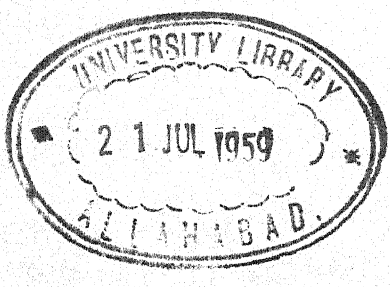
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Biologic Forms of *Albugo candida* (PERS.) KUNTZE on Some Cruciferous Plants

By Makoto HIURA

(Received October 28, 1929)

Introduction

Since ERIKSSON (5) demonstrated clearly the existence of biologic species within *Puccinia graminis* PERS., the biologic specialization in plant disease fungi has become one of the most important problems in plant pathology. In the last two decades, our knowledge of biologic specialization has been greatly advanced (13). Now, it is well known that this phenomenon is very common in various groups of plant disease fungi. It seems quite natural to expect the existence of biologic forms of the causal organism in such cases as when several species or varieties of plants are apparently affected by the same disease. The white rust of crucifers offers a good example. As will be discussed later, some papers concerning biologic forms in *Albugo candida* have been published, but it seems desirable to add more data along this line, because the earlier studies are rather fragmentary.

In the spring and autumn of 1928, the writer carried out a series of inoculation experiments with *Albugo candida* on some cruciferous plants. The results so far obtained are presented in this paper.

Field Observations

As a rule, the disease is most commonly found in April and May in the vicinity of Gifu. It practically disappears during the hot period of the summer months on account of high temperatures, appears again late October to November, and again vanishes in the cold period of the winter because of low temperatures.

As there is an abundance of dew and rain all the year round in this district, the temperature is surely the first limiting factor of the disease. Occasionally, however, the disease may suddenly appear even in the hottest period of the summer, if the temperature becomes suf-

ficiently low; while if the temperature becomes warm enough, the disease may appear even in the coldest period of the winter. The causal fungus is so sensitive to the temperature that we can predict approximately the prevalence and decline of the disease from the temperature, and *vice versa*.

Careful field observations relating to the survival of the fungus over unfavourable periods have been made throughout the year. Living mycelium of the fungus appears to persist in the foliage during both summer and winter, and is therefore probably a factor in the survival of the organism. On the other hand, abundant oospores were found during the summer months and these quite possibly play a part in the reappearance of the disease.

Methods and Materials

Inoculation experiments were performed chiefly in April and May, only a few in November, when the disease was prevalent in the field. Spore suspensions were prepared by dissecting pustules in distilled water with a sterilized knife or needle. An atomizer was used throughout for applying the inoculum. Inoculations were usually made in the evening in order to take advantage of more favourable temperatures. The inoculated plants were kept in moist chambers made of galvanized iron for 13 to 24 hours, and then moved out of doors.

Pustules were first obtained from diseased plants in fields, later from inoculated plants in pots. The names of plants used in this investigation are shown in the following list.

TABLE I

Plants used in the experiments

Japanese name	English name	Scientific name
Daikon	Radish	*Raphanus sativus* L.
Aburana	—	*Brassica campestris* L. subsp. *chinensis* MAKINO
Kabura	Turnip	*Br. campestris* L. subsp. *Rapa* HOOK. f. et ANDS. (*Br. Rapa*)

TABLE I (*Continued*)

Japanese name	English name	Scientific name
Taisai	Pak-choi	*Br. campestris* L. subsp. *chinensis* MAKINO var. *amplexicaulis* MAKINO (*Br. chinensis* L.)
Komatsuna	—	*Br. campestris* L. subsp. *chinensis* MAKINO var. *Komatsuna* MATSUM. et NAKAI
Hakusai	Pe-tsai	*Brassica pekinensis* RUPR.
Kyôna	Pot-herb mustard	*Brassica japonica* SIEB.
Karashina	—	*Brassica cernua* FORBES et HEMSL.
Takana	Chinese mustard	*Brassica juncea* COSS.
—	Rutabaga	*Brassica Napobrassica* MILL.
Kanran	Cabbage	*Br. oleracea* L. var. *capitata* L.
Ryokuyô-kanran	Kale	*Br. oleracea* L. var. *acephala* DC.
Komochi-kanran	Brussels sprouts	*Br. oleracea* L. var. *gemmifera* ZENKER.
Hanayasai	Cauliflower	*Br. oleracea* L. var. *botrytis* L.
Kyûkei-kanran	Kohlrabi	*Br. oleracea* L. var *caulorapa* PASQ.
Nazuna	Shepherd's purse	*Capsella Bursa-pastoris* MOENCH. var. *auriculata* MAKINO

Experimental Data

I. Inoculation Experiments with the Conidia of *Albugo candida* from *Raphanus sativus* L.

In order to determine whether the fungus parasitic on the radish behaves as a distinct biologic form, the conidia from the variety Shima-daikon were inoculated on several species and varieties of cruciferous vegetables. The results are shown in Table II.

TABLE II

Results of Inoculation Experiments with the Conidia from the Radish, Variety Shima-daikon

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Experiment 1 (April 10)						
Shima-daikon (radish)	12 days (Cotyledon)	1	24	+++ ++	Heavy	7 days
Moriguchi-daikon (radish)	,,	1	24	+++ ++	Heavy	7 days
Experiment 2 (April 24)						
Shima-daikon (radish)	7 days (Cotyledon)	2	19	+++ ++	Heavy	9 days
Scarlet globe (radish)	,,	1	19	++	Slight	10 days
Moriguchi-daikon (radish)	,,	1	19	+++ ++	Heavy	9 days
Aburana	,,	1	19			
Experiment 3 (May 2)						
Shima-daikon (radish)	7 days (Cotyledon)	1	15	+++ ++	Heavy	8 days
Takana	15 days (Cotyledon & Leaf	1	15			

TABLE II (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Kyôna	15 days (Cotyledon & Leaf)	1	15			
Shôgoin-kabura (turnip)	,,	1	15			
Benimaru-kabura (turnip)	,,	1	15			
Chirimen-hakusai (pe-tsai)	,,	1	15			
Experiment 4 (May 3)						
Shima-daikon (radish)	7 days (Cotyledon)	1	14	+++	Moderate	7 days
Exhibition (Brussels sprouts)	11 days (Cotyledon)	1	14			
Succession (cabbage)	,,	1	14			
Late giant (cauliflower)	,,	1	14			
Early purple Vienna (kohlrabi)	,,	1	14			
Shirakuki-taisai (pak-choi)	,,	1	14			
Komatsuna	,,	1	14			
Experiment 5 (May 9)						
Shima-daikon (radish)	14 days (Cotyledon & Leaf)	1	14	+++	Moderate	8 days
Aburana	,,	1	14			
Takana	,,	1	14			
Minowase-daikon (radish)	13 days (Cotyledon & Leaf)	1	14	+++	Moderate	8 days

TABLE II (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Kyôna	13 days (Cotyledon & Leaf)	1	14			
Shirakuki-taisai (pak-choi)	,,	1	14			
Shepherd's purse	15-25 cm. tall (Leaf)	1	14			
Experiment 6 (May 11)						
Shima-daikon (radish)	15 days (Cotyledon & Leaf)	1	13	+++	Moderate	7 days
Tokinashi-daikon (radish)	,,	1	13	+++ ++	Heavy	7 days
Scarlet globe (radish)	,,	1	13	++	Slight	8 days
Breakfast (radish)	,,	1	13	++	Slight	9 days
Shôgoin-kabura (turnip)	,,	1	13			
Omitaihei-kabura (turnip)	,,	1	13			
Ko-kabura (turnip)	,,	1	13			
Shirakuki-taisai (pak-choi)	,,	1	13			
Shirakuki-santosai (pe-tsai)	,,	1	13			
Late giant (cauliflower)	,,	1	13			
Aburana	,,	1	13			
Kyôna	,,	1	13			
Komatsuna	,,	1	13			

TABLE II (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Rutabaga	15 days (Cotyledon & Leaf)	1	13			
Chinese mustard	,,	1	13			
Chirimen-hakusai (pe-tsai)	,,	1	13			
Experiment 7 (May 16)						
Shima-daikon	20 days (Cotyledon & Leaf)	2	15	+++ +	Moderate	9 days
Tokinashi-daikon	,,	2	15	+++++	Heavy	8 days
Kameido-daikon	,,	1	15	+++	Moderate	9 days
Moriguchi-daikon	,,	3	15	+++	Moderate	9 days
White icicle (radish)	,,	1	15	++	Slight	9 days
Scarlet globe (radish)	,,	1	15	++	Slight	9 days
Shôgoin-kabura	,,	2	15			
Benimaru-kabura	,,	3	15			
Ko-kabura	,,	2	15			
Early purple Vienna (kohlrabi)	,,	2	15			
Exhibition	,,	2	15			
Komatsuna	,,	2	15			
Chinese mustard	,,	4	15			

TABLE II (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Chirimen-hakusai	20 days (Cotyledon & Leaf	1	15			
Kekkyû-hakusai (pe-tsai)	,,	1	15			
Shirakuki-santosai (pe-tsai)	,,	1	15			
Chifu-hakusai	,,	1	15			
Kyôna (pot-herb mustard)	,,	1	15			
Aburana	,,	3	15			
Shirakuki-taisai (pak-choi)	,,	1	15			
Perfection (broccoli)	,,	2	15			
Late giant (cauliflower)	,,	1	15			
Shepherd's purse	15–30 cm. tall, Leaf	2	15			
Experiment 8 (November 8)						
Shima-daikon	16 days (Cotyledon & Leaf)	1	15	++	Slight	7 days
Takana (Chinese mustard)	,,	2	15			
Karashina	,,	1	15			
Aburana	,,	1	15			

It is quite evident from the above table that the fungus occurring on *Raphanus sativus*, variety Shima-daikon can infect all the varieties of radish tested, but it cannot infect any of the other crucifers used

in these experiments. These results indicate that the parasitic power of the fungus in question is distinctly specialized.

II. Inoculation Experiments with the Conidia of *Albugo candida* from *Brassica campestris* L., subsp. *chinensis* MAKINO

As the *Albugo* fungus was quite common on aburana in our district, trials were made to learn if the spores of *Albugo candida* from aburana can infect other cruciferous vegetables. The same methods as already described were used for the inoculations. The results are presented in Table III.

TABLE III

Results of Inoculation Experiments with the Conidia from Aburana

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Experiment 1 (March 29)						
Aburana	2 months (Leaf)	1	23	+++ +	Moderate	8 days
Chinese mustard	15 days (Cotyledon & Leaf)	1	23			
Shima-daikon (radish)	,,	1	23			
Experiment 2 (April 18)						
Aburana	20 days (Cotyledon & Leaf)	1	24	+++ +	Moderate	8 days
Chinese mustard	,,	1	24			
Experiment 3 (April 24)						
Aburana	7 days (Cotyledon)	2	19	+++ +	Moderate	9 days
Shima-daikon (radish)	,,	1	19			

TABLE III (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Komatsuna	7 days (Cotyledon)	1	19	+++	Moderate	9 days
Shirakuki-taisai (pak-choi)	,,	1	19	+++	Moderate	9 days
Experiment 4 (May 2)						
Aburana	6 days (Cotyledon)	1	15	+++ ++	Heavy	8 days
Chinese mustard	15 days (Cotyledon & Leaf)	1	15			
Chirimen-hakusai (pe-tsai)	,,	1	15	++	Slight	8 days
Shogoin-kabura (turnip)	,,	1	15	+++ ++	Heavy	8 days
Benimaru-kabura (turnip)	,,	1	15	+++ ++	Heavy	8 days
Kyôna	,,	1	15	+++++	Heavy	8 days
Experiment 5 (May 3)						
Aburana	7 days (Cotyledon)	1	14	+++ +	Mederate	7 days
Moriguchi-daikon (radish)	16 days (Cotyledon & Leaf)	1	14			
Exhibition (Brussels sprouts)	,,	1	14			
Succession (cabbage)	,,	1	14			
Late Giant (cauliflower)	,,	1	14			
Early purple Vienna	,,	1	14			

TABLE III (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Experiment 6 (May 9)						
Aburana	14 days (Cotyledon & Leaf)	1	14	+++ +	Moderate	8 days
Shima-daikon (radish)	,,	1	14			
Chinese mustard	,,	1	14			
Minowase-daikon (radish)	,,	1	14			
Shepherd's purse	15–25 cm. tall (Leaf)	1	14			
Experiment 7 (May 11)						
Aburana	16 days (Cotyledon & Leaf)	1	12	+++ +	Moderate	7 days
Ko-kabura (turnip)	,,	1	12	+++++	Heavy	7 days
Shima-daikon (radish)	,,	1	12			
Chifu-hakusai (pe-tsai)	,,	1	12	+++	Moderate	7 days
Shirakuki-santosai (pe-tsai)	,,	1	12	+++	Moderate	7 days
Chirimen-hakusai (pe-tsai)	,,	1	12	++	Slight	8 days
Omitaihei-kabura (turnip)	,,	1	12	+++ ++	Heavy	6 days
Chinese mustard	,,	1	12			
Shepherd's purse	10–25 cm. tall	1	12			
Late giant (cauliflower)	16 days (Cotyledon & Leaf)	1	12			

TABLE III (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Breakfast (radish)	16 days (Cotyledon & Leaf)	1	12			
Scarlet globe (radish)	,,	1	12			
Experiment 8 (May 16)						
Aburana	21 days (Cotyledon & Leaf)	1	15	+++	Moderate	9 days
Komatsuna	,,	1	15	+++	Moderate	9 days
Rutabaga	,,	1	15			
Exhibition	,,	1	15			
Snow White (broccoli)	,,	1	15			
Shima-daikon	,,	3	15			
Kameido-daikon	,,	1	15			
Scarlet globe	,,	1	15			
Tokinashi-daikon	,,	1	15			
Chokurei-hakusai (pe-tsai)	,,	2	15	+++	Moderate	9 days
Chosen-hakusai (pe-tsai)	,,	2	15	+++	Moderate	9 days
Kekkyû-hakusai (pe-tsai)	,,	1	15	+++	Moderate	9 days
Chinese mustard	,,	4	15		Trace (*)	10 days
Succession (cabbage)	,,	2	15			

TABLE III (*Continued*)

Plant		No. of pots	Hours in moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Perfection (broccoli)	21 days (Cotyledon & Leaf)	1	15			
Shôgoin-kabura (turnip)	,,	1	15	+++ ++	Heavy	9 days
Early purple Vienna (kohlrabi)	,,	1	15			
Shepherd's purse	15–25 cm. tall	1	15			
Experiment 9 (May 19)						
Aburana	17 days (Cotyledon & Leaf)	1	15	+++ ++	Heavy	7 days
Shima-daikon (radish)	,,	1	15			
Chinese mustard	,,	1	15			
Experiment 10 (May 29)						
Chinese mustard	17 days (Cotyledon & Leaf)	1	14			
Chinese mustard	24 days (Leaf)	2	14			

According to the above table, the *Albugo* fungus from aburana (*Brassicca campestris*, subsp. *chinensis*) can infect the following species of *Brassica*:

(1) kyôna, *Brassica japonica*.
(2) komatsuna, *Br. campestris*, subsp. *chinensis* var. *Komatsuna*.
(3) turnip, *Br. campestris* subsp. *Rapa*
(4) pak-choi, *Br. campestris* subsp. *chinensis* var. *amplexicaulis*.
(5) pe-tsai, *Brassica pekinensis* RUPR.
(6) Chinese mustard, *Brassica juncea* COSS.

Among these plants, turnips are most severely affected, while pe-tsais are always slightly attacked. As for Chinese mustard, a trace[1] of infection was secured in one case only in eight inoculation experiments.

The fungus cannot infect radish, cabbage, Brussels sprouts, kohl-rabi, cauliflower, broccoli and shepherd's purse.

III. Inoculation Experiments with the Conidia of *Albugo candida* from Chinese Mustard.

A few inoculation experiments were performed with the fungus from Chinese mustard. The results are shown in Table IV.

TABLE IV

Results of inoculation experiments with the fungus from Chinese mustard

Plant		No. of pots	Hours of moist chamber	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Experiments 1 (May 19)						
Shima-daikon	17 days (Cotyledon & Leaf)	1	15			
Aburana	,,	1	15			
Chinese mustard	,,	1	15	+++	Moderate	7 days
Experiment 2 (May 30)						
Aburana	29 days (Cotyledon & Leaf)	2	13		Trace	9 days
Chinese mustard	,,	2	13	+++	Moderate	9 days
Experiment 3 (November 8)						
Aburana	16 days (Cotyledon)	2	15			

(1) The world "trace" used here means a few pustules on one cotyledon.

TABLE IV (*Continued*)

Plant		No. of pots	Hours in moist chamAer	Results		
Variety	Age & Part inoculated			Pustules	Degree of infection	Incubation period
Chinese mustard	16 days (Cotyledon)	1	15	++	Slight	8 days
Karashina	,,	1	15	++	Slight	8 days
Shima-daikon	,,	1	15			

The above table indicates that the fungus on Chinese mustard may be a distinct biologic form, although more extensive inoculations are desirable. The fungus can infect karashina, but cannot infect radish, showing that it differs in its parasitic nature from the fungus on radish. On the other hand, it may be considered that the fungus on Chinese mustard differs from the fungus on aburana, if we remember that the former can infect Chinese mustard severely, but aburana only very slightly, while the latter can infect aburana severely, but Chinese mustard only very slightly.

IV. Morphological Comparison of Biologic Forms of *Albugo candida* on Some Cruciferous Plants

STAKMAN and LEVINE (12) discovered the morphological differences among biological species of cereal rusts, and they established many new varieties. GÄUMANN (6, 7) who studied the genus *Peronospora* found biological as well as morphological differences among the fungi parasitic on the same family, which were formerly considered as one and the same species, and he established many new species. Similar phenomena were also found by BLUMER (1, 2) among certain powdery mildew fungi.

From the same point of view, it seemed desirable to determine whether any morphological differences exist in the *Albugo* fungi parasitic on cruciferous plants. The writer made some measurements of conidia of the fungi on radish, aburana, Chinese mustard, and shepherd's purse. The data are shown in the following table.

TABLE V

Measurements of Conidia of *Albugo candida* on Radish, Aburana, Chinese Mustard, and Shepherd's purse

Host & Date		Length of conidia								
		Class (μ)								
		10	12	14	16	18	20	22	24	Total
Radish (3/23 '28)	Number		2	35	189	206	64	3	1	500
Radish (4/3 '28)	,,			10	95	192	160	41	2	500
Radish (6/28 '28	,,			18	66	172	104	11		371
	Total		2	63	350	570	328	55	3	1371
Aburana (4/19 '28)	Number			12	50	199	201	38		500
Aburana (6/17 '28)	,,			15	86	199	52	3		355
	Total			27	136	398	253	41		855
Chinese mustard (4/17 '28)	Number			5	31	127	119	18		300
Chinese mustard (11/8 '28)	,,		1	10	67	154	95	7		334
	Total		1	15	98	281	214	25		634
Shepherd's purse (4/21 '28)	Number	2	20	48	135	85	10			300

Host & Date		Width of conidia							
		Class (μ)							
		10	12	14	16	18	20	22	Total
Radish (4/3 '28)	Number		7	95	227	149	22		500
Radish (6/28 '28)	,,		10	59	206	88	8		371
	Total		17	154	433	237	30		871

TABLE V (*Continued*)

Host & Date		Width of conidia							
		Classes (μ)							
		10	12	14	16	18	20	22	Total
Aburana (4/19 '28)	Number		9	37	170	216	67	1	500
Aburana (6/17 '28)	,,		9	64	206	71	5		355
	Total		18	101	376	287	72	1	855
Chinese mustard (4/17 '28)	Number		4	33	139	111	13		300
Chinese mustard (11/8 '28)	,,		6	67	180	69	2		324
	Total		10	100	319	180	15		624
Shepherd's purse (4/21 '28)	Number	12	35	136	128	10			321

It is evident from the results shown in the above table that no marked differences in size of conidia are recognizable among the forms on radish, aburana, and Chinese mustard. However, the dimensions of the conidia on shepherd's purse are approximately 2μ less than those of the spores from the three other plants. This difference in size suggests that the fungus on shepherd's purse may be considered as a distinct variety or species, although more extensive measurements are desirable to determine this point.

General Discussion

EBERHARDT (3) was the first to make a biological study of *Albugo candida*. He published his papers in 1903 and 1904 (4). Making several series of inoculation experiments with the conidia from *Capsella*, *Lepidium*, *Brassica* and *Arabis* respectively on various cruciferous plants, he obtained positive results From these results, he concluded that there are probably no biologic species in *Albugo candida*.

It seems noteworthy that EBERHARDT succeeded in infecting cabbages with the fungus from turnips, while the writer failed to infect cabbages with the fungus from aburana. which readily infects turnips. This suggests indirectly that there may be two biologic forms of the fungus occurring on turnips, although further studies are necessary to determine this point.

Later, MELHUS (8) published the results of detailed investigations concerning the physiology and biology of *Albugo candida*. His elaborate experiments proved that low temperatures play an important role in both the germination of conidia and infection. Much attention was also given to biological species of *Albugo candida*. He used the conidia from radish as inoculum throughout its experiments. Infections were secured on *Raphanus caudatus* and all the varieties of *Raphanus sativus*, but not on the other crucifers tested, with the exceptions of white mustard and cabbage, on which slight infections were obtained. These results of experiments suggest the existence of specialization in *Albugo candida*, as indicated in the following statement by MELHUS:

"My results show that it is possible to inoculate several other crucifers with the spores of *Cystopus* obtained from the radish, which tends to preclude the possibility of so called physiological species in accordance with EBERHARDT'S conclusions; yet it may well be that limited specialization exists when further cross inoculations with the spores from other hosts have been made" (p 76).

The results of the writer's inoculation experiments with the fungus from the radish coincide in the main with those of MELHUS. As has been set forth, all the varieties of *Raphanus sativus* tested in the present studies were infected by the fungus from the variety Shimadaikon, but turnip, rutabaga, shepherd's purse etc. were not infected. Only one point of difference is to be found, namely that the writer failed to infect the cabbage with the fungus from radish, while MELHUS succeeded. This, however, may be explained in part by the fact that the writer did not inoculate so many varieties as did MELHUS. Generally speaking, the fungus on radish, tested by the writer is seemingly identical with the fungus studied by MELHUS, but at present, the data are too few to establish this view with certainty.

In 1920, PAPE and RABBAS (9) reported that with the conidia from *Capsella bursa-pastoris*. infection was not secured on *Brassica Napus*, *Raphanus sativus*, *Raphanus oleiferus*, *Cheiranthus Cheiri*, *Sinapis arvensis*, and *Sisymbrium sinapistrum*. This specialization of the fungus on shepherd's purse is also possible of prediction from either

the results of MELHUS or those of the writer. However, whether the fungus on shepherd's purse can infect any other crucifers is still a question. According to EBERHARDT, the fungus on shepherd's purse can attack various other crucifers which were not used by PAPE and RABBAS in their inoculation experiments.

From the above mentioned considerations, there is no doubt about the existence of specialization in *Albugo candida*, although the degree of specialization is not so great as in the cereal rusts. If more extensive inoculations are made, the range of infection of the biologic forms described may prove to be more extended. However, this does not necessarily disprove the existence of specialization in *Albugo candida*. It concerns only the range of infection.

Thus far, four distinct biologic forms of the fungus have been found on radish, turnip, Chinese mustard, and shepherd's purse respectively. It seems highly possible that more biologic forms may be added by further cross inoculations on many other crucifers. Several distinct biologic forms have been recently demonstrated for the species, *Albugo tragopogonis* (10).

In the present studies, the incubation period[1] ranged from 7 to 10 days. It seems noteworthy that the incubation period was 1 to 2 days longer in less susceptible varieties (See Tables). This is in accordance with the observations of STAKMAN (11) on *Puccinia graminis*, and of others.

Summary

(1) In order to study the existence of biologic forms in *Albugo candida*, the fungi occurring on radish, aburana, and Chinese mustard were inoculated on various varieties of cruciferous plants.

(2) It has been proved that the fungus on radish can infect all the varieties of radish, but cannot infect any other crucifers tested; that the fungus on aburana can infect kyôna, komatsuna, turnip, pak-choi, pe-tsai, Chinese mustard, and its original host with different degrees of severity, but cannot infect any other crucifers tested; and that the fungus on Chinese mustard can infect karashina, aburana, and its original host with different degrees of severity, but cannot infect radish. From these results the writer has concluded that these three fungi are distinct biologic forms.

(1) The term "incubation period" used here means the period from inoculation to the first appearance of pustules.

(3) No marked differences in size of conidia have been recognized among the fungi on radish, aburana, and Chinese mustard, but the fungus parasitic on shepherd's purse bears smaller conidia than the three other forms.

(4) The incubation period ranged from 7 to 10 days, and was 1 to 2 days longer in less susceptible varieties of hosts.

The present studies were carried out in the laboratory of Plant Pathology of the Gifu Imperial College of Agriculture with the assistance of Mr. H. KANEGAE, to whom the writer wishes to express his heartiest thanks. He is also indebted to Mr. S. KARIYA in various ways. September 30, 1929.

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A New Species of *Urocystis* on *Convallaria majalis* L.

By Kogo TOGASHI and Fusaji ONUMA[1]

With 1 Text-figure

(Received December 15, 1929)

In the beginning of June of last year (1928), the attention of the writers was called to an unfamiliar disease of *Convallaria majalis* at a grassy place half-way up Mt. Himekami (1124.5 m.) in the Province of Rikuchu. The affected plants were stunted in various degrees and their color more or less changed to a yellowish green. On the leaf-blades and sheaths of the diseased plants, many large groups of minute grayish pustules along the parallel veins were noticed at a glance. Bringing them into our laboratory, the writers ascertained that this causal fungus was a species of the genus *Urocystis*.

The genus *Convallaria* belongs to the subfamily Asparagoideae of Liliaceae. As far as the writers are aware, three species of *Urocystis*, *U. Jaapiana* SACC., *U. Trillii* S. JACKSON and *U. Colchici* (SCHLECHT.) RABENH., parasitic upon the different members of the subfamily, have been described up to the present. Naturally, we compared them with our fungus in question and recognised that the last one appears to have a resemblance to ours to some extent.

The last species *Urocystis Colchici* was first described in 1826 by SCHLECHTENDAHL (Fungorum novorum et ensufficienter descriptorum illustratio, Linnaea, Vol. 1, p. 241) under the name of *Caeoma Colchici*, based on a specimen of *Colchicum autumnale* collected in Hercynia. During some of the decades afterwards, it was given different names by various authors and finally distributed in 1861 by RABENHORST in his Fungi Europaei as No. 369, with its proper generic name, *Urocystis*. In subsequent publications, however, the extent of the host plant range has been disputed by the authors without reaching an agreement. WINTER (1884) treated all the forms of *Urocystis* found on *Convallaria*

(1) The writers wish to express their hearty thanks to Prof. S. ITO, Hokkaido Im perial University for his kind advice and criticism.

Polygonatum (=*Polygonatum officinale*), *Ornithogalum umbellatum*, *Scilla bifolia*, *Allium rotundum*, *A. Cepa*, *Muscari comosum*, *M. racemosum*, and *Colchicum autumnale* as one species of *U. Colchici*. DE TONI (1888) separating this species from *U. Cepulae* FROST, *U. magica* PASS. and *U. Ornithogali* KOERN. recorded its host plants as follows: *Allium rotundum*, *Colchicum autumnale*, *Muscari comosum M. racemosum*, *Paris quadrifolia*, and *Scilla bifolia*. OUDEMANS (1919) in his

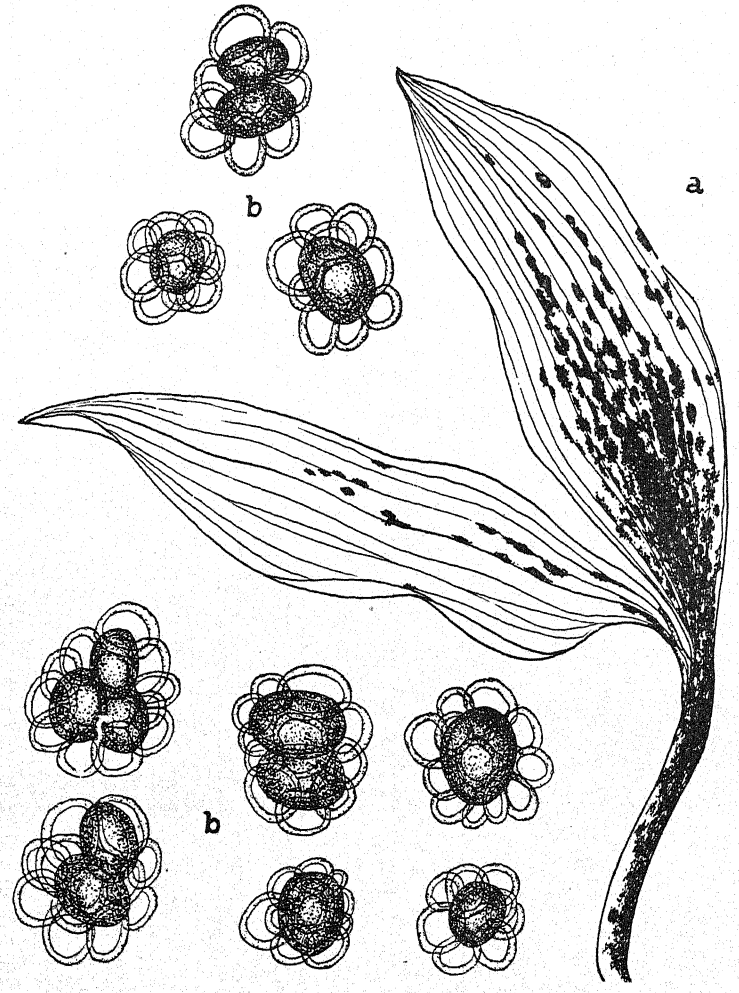


Fig. 1. a. Affected plant (×1/1).
b. Spore-balls of *Urocystis Miyabeana* n. sp. (×720).

"Enumeratio Systematica Fungorum" mentioned the following plants as its hosts: *Bumbocodium vernum*, *Colchicum autumnale*, *Allium nigrum*, *A. rotundatum*, *A. subhirsutum*, *Muscari comosum*, *M. racemosum*, *Ornithogalum umbellatum*, and *Scilla bifolia*.

CLINTON (1906), on the other hand, reported only three species as the North American host plants of *U. Colchici*, viz. *Salomonia commutata* (=*Polygonatum giganteum*), *Vagnera amplexicaulis* (=*Smilacina amplexicaulis*), and *V. stellata* (=*S. stellata*). SCHELLENBERG (1911) pointed out that there are distinct morphological differences among the forms of *Urocystis* parasitic on Liliaceous plants and he divided the forms into five species, remarking that "Als Nährpflanze ist vor allem *Colchicum autumnale* L. bekannt geworden. In wieweit die auf anderen Liliaceen angegebenen *Urocystis*-Formen hierher gehören, bedarf der weiteren Untersuchung." His classification is as follows:

U. Colchici (SCHLECHT.) RABENH. on *Colchicum autumnale*

U. Ornithogali KOERN. on the species of *Scilla*, *Ornithogalum* and *Muscari*

U. Cepulae FROST on *Allium Cepa*

U. Allii (BELTRANI) SCHELLENB. on *Allium oleraceum*, *A. hirsutum*

U. magica PASS. on *Allium magicum-nigrum*.

Considering the generic relationship of the host plants, SCHELLENBERG'S classification appears very desirable and proper, though he did not comment clearly on this point, because the genus *Colchicum* belongs systematically to the subfamily *Melanthioideae*, the genera *Scilla*, *Ornithogalum* and *Muscari* to the subfamily *Lilioideae* and the genus *Allium* to the subfamily *Allioideae*. As to the pathogenicity of *Urocystis* occurring on the different species of the genus *Allium*, ANDERSON (1925) made a study with the result of indorsing the opinion of SCHELLENBERG. After making the above mentioned review on *U. Colchici* and its allies, especially on the generic connection of the host plants, we went on to compare the morphological character of the fungus we discovered to *U. Colchici*, as well as to the related species. The result is given in the following table.

TABLE. Essential characters of our fungus and the related species of *Urocystis* given by various authors

Fungi	*U. Colchici*	*U. Jaapiana*	*U. Trillii*	Our fungus
Authors	SCHELLENBERG, 1911	SACCARDO, 1915; TROTTER, 1925	JACKSON, 1920; TROTTER, 1925	TOGASHI & ONUMA, 1929
Sori	In leaves, deeply embedded, linear along the veins, easily powdered, spore-masses dark brown	In young shoot, inflated, deformed, ruptured longitudinally, black, powdery	Hypophyllous, or caulicolous, round or oval, 0.5–1.5 mm., scattered, commonly gregarious in circular groups, opening tardily, spore-masses purple black	In leaves and sheaths, verruciform along the veins, forming an irregular large patch, grayish black, long covered, spore-masses black brown
Spore-balls	Globose to oval, 20–30 μ	Irregularly spherical, 16–19μ	Chestnut brown, globoid, 24–50 μ or ellipsoid, 50–70× 30–40 μ	Subglobose or ellipsoidal, 17–37× 15–34μ, generally 20–30×19–27 μ
Spores	1–2, rarely 3, oval, rarely globose, 14–20 μ, loosely surrounded by the sterile cells, membrane dark brown	1–3, mostly 1, globose, 11–12 μ, on one side depressed, tawny	Subgloboid, ovoid or polygonal, 3–20, rarely 1–2, mostly 10–15 μ, membrane 2–3 μ, chestnut brown	Globose or ovoid, 1–4, mostly 1–2, dark brown, 11–22×10–18 μ, generally 13–17×11–16 μ, closely surround by the sterile cells
Sterile cells	Variable in diam., 6–12 μ, membrane light brown	Numerous, hemispherical, 4.5–6 μ, hyaline	Subgloboid or polygonal, 5–9 μ, membrane golden brown, 1–1.5 μ	Subglobose, ellipsoidal, light brownish yellow, 6–25, 6–16×5–14 μ, generally 8–11 ×6–10 μ

U. Colchici produces a linear pustule on the leaves which eventually ruptures the epidermis with a longitudinal slit, disclosing a powdery mass of spore-balls, while the pustule of our fungus is at first minute, and verruciform, at length occupies a greater part of the leaf, forming a gregarious irregular large patch and remaining long covered by the epidermis. Further, in a spore-ball the central spores of our fungus are closely surrounded by more numerous sterile cells than those of *U. Colchici*, although there are no conspicuous differences in the dimensions of the spores and sterile cells of each.

These characteristics and the diversity of the host plants clearly show that our fungus is distinct from *U. Colchici*.

As seen in the above table, it is evident that our fungus is also clearly different from *U. Jaapiana* and *U. Trillii* that no further explanation is needed.

In conclusion, the writers wish to treat the fungus under consideration as a new species, proposing for it the name *Urocystis Miyabeana* in honor of Dr. Kingo MIYABE, Emeritus Professor of the Hokkaido Imperial University.

Urocystis Miyabeana Togashi et Onuma n. sp.

Sori in the leaves and sheaths, grayish black, verruciform, showing through on both sides, at first 0.7—1.0 mm. in diameter, gregarious in irregular forms, at length occupying a greater part of the leaf, long covered by the epidermis but finally rupturing this and disclosing a granular blackish brown spore-mass; spore-balls deeply embedded in the mesophyll, subglobose or ellipsoidal, 17–37 × 15–34μ, generally 20–30 × 19–27μ; sterile cells subglobose, ellipsoidal, rarely discoidal, light brownish yellow, closely surrounding the spores, usually 6–25 in a spore-ball, 6–16 × 5–14μ, generally 8–11 × 6–10μ, membrane 1–1.5μ thick; spores globose or ovoid, mostly flattened where they are in contact, 1–4, chiefly 1–2 in a ball, dark brown, 11–22 × 10–18μ, generally 13–17 × 11–16μ, membrane smooth, 2μ thick.

Hab. on leaves and sheaths of *Convallaria majalis* L. (*Kimikageso.*) Prov. Rikuchu: Mt. Himekami, June 1 & 15, 1928, K. TOGASHI and F. ONUMA.

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Über den Ursprung des dreigliedrigen Quirls von *Gardenia jasminoides*, ELLIS

Von Toichi ASAI

Mit 3 Textfiguren

(Eingegangen am 8. Januar 1930)

Die Blattstellung ist eine angeborene Eigenschaft, die durch die Umgebung schwerlich verändert werden kann; so wird sie öfters als eines der wichtigen Merkmale der Gattung und Familie der Pflanzen betrachtet. Es gibt den Fall, dass zwei oder mehrere Laubblätter aus ein und demselben Knoten des Sprosses entspringen, aber noch häufiger in der Natur kommen die schraubig gestellten Blätter an dem Umfang des Zweigs vor.

Bei den höheren Lebermoosen treten die Blättchen mit der Schieferstellung in zwei Zeilen auf; ferner sind die Blättchen der Laubmoose in den meisten Fällen spiralig an dem aufrechten Stämmchen gestellt. Die Blattstellung der Farnpflanzen ist im allgemeinen schraubig, ausgenommen *Salvinia*, *Equisetum* und derartige besondere Gruppen. Bei Samenpflanzen zeigen auch die Gymnospermen und die Monokotylen alle mit einigen Ausnahmen fast das gleiche Stellungsverhältnis der Blätter. Bei Dikotylen, wenn die gegen- und quirlständigen Merkmale verhältnismässig gut hervortreten, nehmen doch die Familien, welche die Arten mit solchen Merkmalen enthalten, nur etwa 23 Proz. der gesammten Zahl ein, und diejenigen mit dem quirlständigen Merkmal übersteigen nicht 4 Proz., während die Zahl der die gegenständigen Blätter tragenden Gruppen an 16–17 Proz. reicht.[1]

An *Gardenia jasminoides* setzen die gegenständigen Blätter sich gewöhnlich kreuzweise an, aber wir bemerken auch öfters bei in dem Wald wildwachsenden Pflanzen, dass der dreigliedrige Quirl in sechs Zeilen an dem Umfang des Zweigs aufgestellt ist. Bei einigen Arten von *Salix*, *Rhamnus*, *Veronica* und *Dahlia* stellen sich die Laubblätter an ein und demselben Stengel oder Zweig, teilweise quirlig, teilweise

(1) Die quirlständige Form wird sehr häufig an den Wasserpflanzen und den Schuppenblätter tragenden Arten beobachtet.

TABELLE I

Verhältnis der Blattstellung und Familien bei den Dikotylen(1)

Blattstellung	Familienzahl	
	Choripetalae	Sympetalae
wechselständig	135	30
wechsel-, selten gegenständig	3	4
wechsel- oder gegenständig	14	3
meist gegenständig	7	0
gegenständig	17	6
wechsel-, selten gegen- oder quirlständig	1	0
wechsel-, gegen- oder quirlständig	0	3
gegen- oder quirl-, selten wechselständig	0	1
wechsel-, selten quirlständig	1	0
gegen- oder quirlständig	6	5
quirlständig	3	0

schraubig auf. Als ein gewöhnliches Merkmal von Rosaceen kommen die Laubblätter wechselständig vor, aber selten im oberen Teil gegenständig. Bei Selagineen sind die Laubblätter wechselständig, nur das unterste ist bisweilen gegenständig und bei Pedaliaceen gegenständig oder nach obenhin wechselständig.(2) In diesen Fällen geht ein Blatt unzweifelhaft durch die Umstellung von einem Knoten nach dem

(1) A. ENGLER: Syllabus der Pflanzenfamilien, 1912; F. THONNER: Anleitung zum Bestimmen der Familien der Phanerogamen, 1891.

(2) A. KERNER: Pflanzenleben, Bd. I, 1905, S. 383.

anderen über; also die gegenständigen Blätter halten sich auseinander, ohne sich in ein und derselben Höhe des Zweigs genau aufzustellen. Aber nun möchten wir auch mit anderem Gedanken über die Blattstellung der Pflanzen hierunter erklären, dass die Blattstellung öfters durch die Spaltung des Laubblattes in die gegen- oder quirlständige übergeht: die gegen- kann von der wechsel-, die quirl- von der gegenständigen abgeleitet werden, und diese Tatsache habe ich gelegentlich vor einigen Jahren an *Gardenia jasminoides* beobachtet.

Ich habe einen Stock, welcher von der Keimlingszeit in dem Topfe von mir besorgt wurde. Er sieht äusserlich aus, als ob zwei Stöcke, deren Blattstellung ganz von einander verschieden ist, nahe beieinander gewachsen wären. Bei näherer Betrachtung kann man aber bemerken, dass er an dem Grund in zwei Teilen, ein Zweig mit den gegenständigen Blättern und der andere mit dem dreigliedrigen Quirl, abgezweigt und übrigens der Stammscheitel der jungen Pflanze schon vorher verdorben war (Fig. 1). Dann schien es mir, dass die Verzweigung dieses Stocks ein abnormaler Fall sei, der auf der Verletzung des Stammscheitels beruhte. Ein Jahr danach fand ich ein anderes Exemplar: ein Stock mit dreigliedrigem Quirl unter den einst im Baumschatten fortgepflanzten hatte etwa 30 cm über dem Boden rechts und links abgezweigte Sprosse; damit kreuzend entsprangen diejenigen mit den gegenständigen Blättern nahe über den letzteren nach vorn und hinten, und die Entwicklung der Hauptachse hörte infolge von Verletzung, wahrscheinlich dem Raupenfrass, ganz auf.



Fig. 1. Junger *Gardenia*-Stock mit Zweigen verschiedener Blattstellung.

Nun wurde es mir aus obigen zwei Beobachtungen klar, dass die Sprosse mit den gegenständigen Blättern unzweifelhaft aus den latenten Knospen entspringen, wenn der Scheitel der Hauptachse einer dreiquirlige Blätter tragenden *Gardenia*-Pflanze verletzt wird. Entstehen die Sprosse mit den gegenständigen Blättern auch auf dieselbe Weise wie in der Natur, wenn die Zweige mit dem von Natur dreigliedrigen Quirl künstlich abgeschnitten werden? Und, werden die Zweige mit den gegenständigen Blättern am Scheitel abgeschnitten, ist die Stellung

der aus den latenten Knospen abgeleiteten Blätter immer gegenständig, wie erwartet? Im August 1926 habe ich je einige Zweige aller zehn Stöcke mit den quirligen Blättern und die der andern zehn mit den gegenständigen nahe über dem dritten Glied von dem Scheitel abgeschnitten. Dann trieben die latenten Knospen in den meisten Fällen aus der Blattachse, häufig nahe darüber, eine, bisweilen gegenseitig, oder gar keine Knospe. Für Ende März des nächsten Jahres ist die angestellte Folge der Versuche in der folgenden Tabelle zusammengefasst.

TABELLE II

Verhältnis der neuen Sprosse mit den gegen- und quirlständigen Blättern, die aus den abgeschnittenen Zweigen auftreiben

(1) bei den gegenbeblätterten Stöcken:

Nummer der Stöcke	Zahl der abgeschnittenen Zweige	neue Sprosse mit gegenständigen Blättern	neue Sprosse mit quirlständigen Blättern
1	5	0	0
2	4	4	0
3	4	5	0
4	3	2	0
5	2	0	0
6	5	3	0
7	4	4	0
8	3	0	0
9	4	4	0
10	3	2	0

TABELLE II (Fortsetzung)

(2) bei den quirlbeblätterten Stöcken:

Nummer der Stöcke	Zahl der abgeschnittenen Zweige	neue Sprosse mit gegenständigen Blättern	neue Sprosse mit quirlständigen Blättern
1	3	4	0
2	3	1	0
3	3	0	0
4	2	0	0
5	3	1	2
6	4	4	0
7	3	2	0
8	3	0	0
9	5	4	0
10	4	3	0

Bei Zweigen mit gegenständigen Blättern wurde kein neuer Spross an 3 von den 10 geschnittenen Stöcken gesehen. Aber 24 Sprosse sind aus 37 abgeschnittenen Zweigen im Verhältnis von 65 Proz. in der Nähe jedes Glieds entsprungen und die Blattstellung war natürlich in allen Fällen immer gegenständig. Bei den Stöcken mit den quirligen Blättern sind die Sprosse in etwa gleichem Verhältnis (64 Proz.) entwickelt, und die Laubblätter an dem neuen Spross waren auch fast alle gegenständig, wie ich zuerst annahm. Nur ein Stock mit quirligen Blättern, welcher sehr in die Augen fiel, hatte je einen Spross an jedem von den 3 abgeschnittenen Zweigen, und an dessen Sprossumfang verteilten sich die Laubblätter, nicht nur zwei-, sondern auch dreigliedrig. Die Laubblätter an einem von drei neuen Sprossen stellten sich gegenständig wie gewöhnlich, dagegen in den übrigen Fällen quirlständig auf. Aber die Verteilung der quirligen Blätter ist etwas von dem

normalen dreigliedrigen Quirl verschieden; ein von 3 Laubblättern an demselben Knoten ist insbesondere viel grösser als die anderen und sie sind nicht mit einer bestimmten Divergenz verteilt, vielmehr sind 2 einander nahestehende kleine Blätter zusammen einem grösseren Blatt gegenüber gegenständig (Fig. 2, oben).

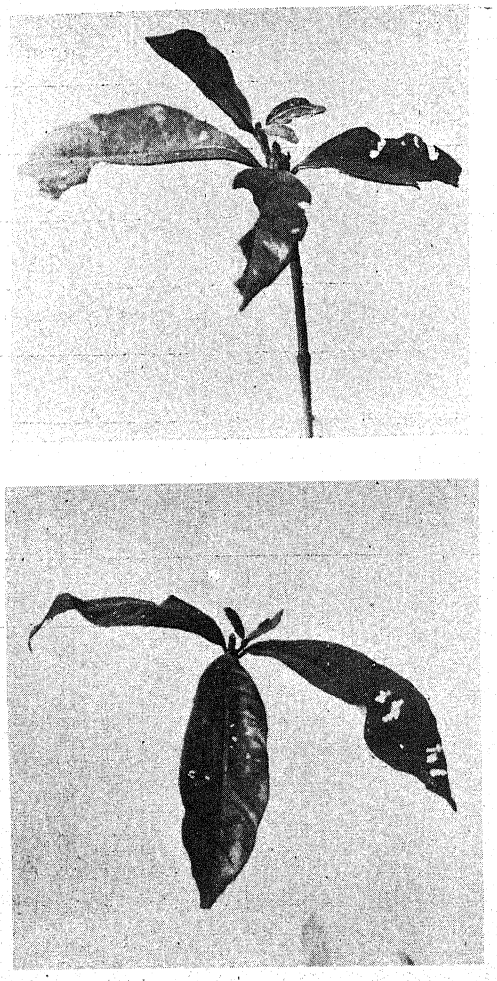


Fig. 2. Zwei aus den abgeschnittenen Zweigen eines Stocks getriebenen Sprosse; oben der quirlblätterige, unten der gegenblätterige Spross.

Unter wildwachsenden *Gardenia jasminoides*-Stöcken finden wir diejenigen mit dreigliedrigem Quirl in Menge auf dem Hügel Tatsuda bei Kumamoto; an diesen habe ich einst Zweige, welche an einem oder einigen Knoten gegenständige Blätter tragen, bemerkt. Danach aber sind solche Zweige dort von mir überall gesehen worden, und sogar kommt in der Natur auch nicht selten der dreigliedrige Quirl an einem oder einigen Knoten des Zweigs mit gegenständigen Blättern vor. Im äussersten Falle setzen die Laubblätter sich an ein und demselben Zweig gegen- und quirlständig nach dem andern alterniert an (Fig. 3). Aber wir können an dieser Blattstellung mit Leichtigkeit bemerken, dass ein Blatt des Quirls an einem Knoten häufig etwas grösser als die übrigen ist, und die nahe beieinander stehenden kleinen Blätter dem andern Laubblatt sich entgegensetzen und diese Laubblätter in die der benachbarten Knoten kreuzweise übergehen.

Ich darf hier noch über eine andere Beobachtung, wo das quirlige Merkmal auch bisweilen an den Blättern des Keimlings von *Gardenia jasminoides* vorkommt, berichten. Im Frühling 1929 säte ich Samen, welche ein wildwachsender gefülltblütiger Stock[1] liefert, in dem Garten aus. Aus 143 Körnern einer Schote wurden 68 Keimlinge erhalten; 3 Stöcke von diesen waren quirlblätterig, und zwar waren die Keimblätter selbst ungewöhnlich dreigliedrig; allerdings zeigen sie nur wenig Verschiedenheit in der Blattgrösse, wie man sie in dem Quirl eines erwachsenen Zweigs häufig sieht.

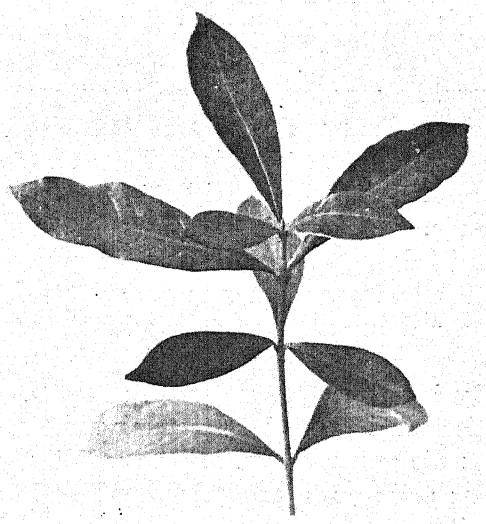


Fig. 3. Zweig mit gegen- und quirlständig alternierend angeordneten Blättern.

Wie bereits erwähnt, ob nun ein quirl- oder gegenblätteriger Zweig abgeschnitten wird, ist die Blattstellung an dem neu getriebenen Spross fast immer gegenständig; und obgleich der Quirl an den abgeschnittenen quirlblätterigen Zweigen nur selten ausgebildet wird, stellen zwei kleinere Laubblätter des Quirls sich einem andern gegenüber gegenständig, ohne sich in demselben Abstand zu verteilen. Überdies findet man in der Natur zuweilen den Quirl an einem oder einigen Gliedern des gegenblätterigen Zweigs, wie ich ihn an den Sprossen aus dem abgeschnittenen Zweig gesehen habe. Aus diesen Tatsachen möchte ich für möglich halten, dass der dreigliedrige Quirl bei *Gardenia jasminoides* daraus entsteht, dass eines von dem Blattpaar an einem Glied sich in zwei teilt. In der gleichen Weise muss es beim viergliedrigen Quirl auch wohl geschehen, jedoch hatte ich keine Gelegenheit, ein solches Exemplar in der Natur zu treffen, obwohl es möglicherweise entstehen könnte.

(1) T. ASAI: Über die wildwachsenden gefülltblütigen Stöcke von *Gardenia jasminoides*, ELLIS. Japan. Jour. of Bot., **4**, No. 4, 1929.

Wie soeben erwähnt, sind die Keimblätter bisweilen dreigliedrig und ich kann aus Erfahrung darüber sprechen, dass die auf dem oben genannten Hügel Tatsuda gesammelte Schote in einem kleinen Prozentsatz, immer die Keimlinge mit quirligen Blättern lieferte. Obwohl diese Eigenschaft freilich etwas gründlich bei *Gardenia jasminoides* sei, wenn die Zweige mit quirligen Blättern absichtlich abgeschnitten werden, treiben sie quirlblätterige neue Sprosse nur in sehr seltenem Falle. Überdies ist es aus der Blattstellung an diesem dreigliedrigen Quirl deutlich, dass er von den gegenständigen Blättern eines Glieds herkommt; und an den gegenblätterigen Zweigen kann man keinen Quirl durch die Verletzung des Scheitels künstlich ausbilden. In der Natur sehen wir auch zuweilen die gegenblätterigen Glieder an den Zweigen des quirlblätterigen Stocks. Kurz, es gibt zwei auffallende Merkmale in der Blattstellung von *Gardenia jasminoides*: eines davon ist die gegenseitige, welche als die allgemeine wohl bekannt ist; als das andere soll das quirlblätterige Merkmal stehen, jedoch macht es seinen Einfluss auf die Blattstellung im Vergleich mit dem gegenseitigen Merkmal schwer. Obgleich auch das dreiquirlige Merkmal an den Stöcken auffallend zu Tage tritt, kommt das erste, mächtigere, gründlichere Merkmal leicht zum Vorschein, wenn der Scheitel des Zweigs durch gewisse äussere Verhältnise, verletzt wird. Dann möchte ich auf den phylogenetischen Ausgang bei dem Merkmal schliessen, dass das erste die Grundform der genannten *Gardenia*-Art sei, und das zweite sei davon sekundär neu in der Entwicklung dieser Art entstanden. Das quirlblätterige Merkmal tritt durch innere Ursachen manchmal auf, allein es mag vielleicht eine rezessiv vererbbare Eigenschaft sein, indem sie von dem gegenseitigen immer bedrängt wird. Sobald die Gelegenheit dazu geboten wird, taucht die Grundform immer leicht auf, selbst wenn der Stock die Ausbildung des dreigliedrigen Quirls schon angenommen hatte.

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The Life-History and Physiology of *Synchytrium fulgens* SCHROET., with Special Reference to its Sexuality

By Shunsuke KUSANO

With 19 Figures in the Text

(Received January 30, 1930)

I. Introduction

Since olden times an attention has been directed towards the question of sexuality in *Synchytrium*. In the "Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien, 1884," DE BARY (p. 181–183) expressed on reasonable grounds a view as to the most probable occurrence of sexuality. But it has not been proven to be a fact until the appearance of CURTIS' excellent paper in 1922 on *S. endobioticum*, in which the process of the zygote formation by motile isogametes was described in detail. In this first record of sexuality in *Synchytrium* we see that the sexual action is very labile; the swarm cell may act sometimes like an asexual zoospore and sometimes like a sexual gamete. In the light of sex differentiation an investigation into the nature of such a primitive sexuality seems to promise some results of general interest.

In taking up *Synchytrium fulgens* as the object of study much weight has been laid upon the problem of sexuality. Extensive observations and experiments, chiefly made in this connection, gave us an opportunity to make its life-history much clearer and to give some light on our knowledge of its physiology. The present report embodies all the chief results obtained relating to these subjects.

The account to be given is the outcome of studies covering several years on living materials. As it seemed that certain facts thus obtained need support by cytological evidences, the rich material accessible during the work was submitted to cytological investigations. I was thus able to follow out the nuclear features throughout the life-history of the fungus with satisfaction. In the present paper only

some of the results will be referred to, reserving a full account for future communications.

No other member of the genus has been investigated so extensively and intensively as *S. endobioticum*, a well-known fungus which is the cause of the dreadful wart disease of potato, and indeed papers treating practical and scientific problems about this fungus have amounted to a considerable number. A review of the very important literature thus furnished is not attempted here, as reference will often be made at proper places in the present report.

II. Description of the Fungus

Since our knowledge of the present fungus is very inadequate, I proceed to present at the outset its morphology and life-history in an outlined form, thinking that it makes what is recorded in the present paper more comprehensive than else.

The fungus is parasitic on *Oenothera Lamarckiana* and *O. biennis*. It invades the epidermal cells of their leaves and stems, and with a rapid growth develops into either the gametangium sorus or the resting cell.

The gametangium sorus: The fungus body (prosorus) developing into the sorus is already orange-coloured at an early stage. After it has grown to occupy the entire cavity of an enlarged host-cell, its whole body undergoes segmentation and is transformed into a gametangium sorus. The sorus is generally spherical or often ovoidal. The medium-sized one attains a diameter of ca. 100 μ[(1)] and contains about 50 gametangia which are spherical or ovoidal, measuring 21–37 μ in diameter (maximum $28 \times 40\ \mu$). Upon maturation of the sorus the wall of the host-cell is broken at its exposed portion and a mass of gametangia is squeezed out of the sorus. The freed gametangia are at first somewhat moist, being coherent and adherent as well, but sooner or later they become pulverous on drying and are dispersed, like some uredospores or aecidiospores, over the surface of the host, as has often been noticed by previous authors.

The planogamete is pear-shaped, attaining a length of 5 μ, containing an orange oil-drop, and being provided with a long posterior cilium. All gametes are morphologically similar.

(1) A single host-cell often contains two sori appearing as a single sorus having a diameter of 180 μ and containing about 150 gametangia.

The resting cell: While the prosorus is becoming recognizable as an orange spot with the naked eye, another fungus body developing into the resting cell can be distinguished as a smaller milky spot. When full grown it is provided with a thick wall, consisting of three layers, the brownish epispore, the hyaline mesospore, and the indistinct endospore. On the surface of the wall are compactly deposited brownish globules derived from the host-cell, so that the resting cell gives a warted appearance.

The resting cell is spherical, rarely ovoidal, single or 2–3 in each host-cell. The diameter ranges from 35 to 90 μ, being 50–60 μ in predominance. On germination the content, after turning orange in colour, migrates into a spherical sac which bulges out through a small pore on the wall. The sac is later transformed into a gametangium sorus, not exceeding in size the resting cell. The gametangia and gametes derived from them show no morphological differences from those already mentioned.

For distinguishing two kinds of the gametangium sorus and its derivatives, the gametangium and the gamete, it is proposed to prefix "winter" to those originating from the resting cell and "summer" to those of the other kind.

The gamete is capable of parthenogenetic development; while a zygote produced by two gametes gives rise to the resting cell, an uncopulated gamete directly develops into the summer sorus. The winter and summer gametes behave quite similarly in this respect, so that the winter gametes may give rise to the generation of either the summer sorus or the resting cell.

III. The Germination of the Summer Gametangium

1. The Process

The content of the mature gametangium is homogeneously orange-coloured. On germination the colouring matter appears as if undergoing a process of condensation or precipitation, being differentiated as fine granules from the hyaline matrix. With the progress of germination the granules are further condensed into small globules of a deeper colour, making thus the content of the gametangium much clearer. At this stage the segmentation of the cytoplasm into gametes seems to be completed. By absorbing water the gametangium is much swollen, and a hyaline space appears between its wall and the conglom-

erate contents. In all probability the space is occupied by imbibition water. On account of an increased internal pressure the gametangium wall projects outwards at several places. After some minutes the gametangium ruptures at one of the projected places, and, if the slit thus produced is large, a number of gametes are released at once; if small, each gamete escapes one by one. Immediately after a few gametes are freed, a jerking movement is observed in those remaining in the gametangium, meanwhile they escape through the slit in succession, leaving the gametangium vacant usually in a few minutes.

The empty gametangium shrinks and resumes an original polyhedral form. The slit is always found at the center of a polygonal section of the wall. It shows without doubt that this portion is least resistant towards the internal pressure exerted during germination.

2. Relation to Temperature

The rate of germination varies considerably according to the temperatures, to which the medium is exposed. In general, at a temperature of 20°C. some gametangia germinate in about one hour and a half, and others follow in rapid succession. Within the range of 18°–22°C. the rate is nearly the same. At higher or lower temperatures, not only is the germination delayed more or less, but the rate widely diverges from each other in individual gametangia. Some of the experiments showing this fact are given in the following.

1. Gametangia taken from the same diseased patch were germinated at several temperatures. The results are tabulated below (Table I).

TABLE I

Temp. / Hours	9°–10°C.	13°C.	18°–20°C.	24°C.
2	No germination	No germination	Gametes crowded; a large number of them at rest	Gametes crowded; a large number of them at rest
5	A small percentage of germination	A large percentage of germination; majority of gametes at rest	A great majority germinated; active gametes very few	A great majority germinated; active gametes very few
7	More than 50% germinated; active gametes yet abundant	Most gametes at rest	All gametes encysted	All gametes encysted

In this experiment it is shown that a temperature as low as 9°–10°C. causes a considerable delay of germination, though it does not absolutely inhibit it. At 24°C. the process of germination is essentially similar to that at 18°–20°C.

2. Hanging drops with gametangia were placed in a room of 8°–9°C. As a control other drops were kept at a temperature of 17°C. (see Table II).

TABLE II

Temperature / Hours	8°–9°C.	Control
1 2/3	No germination	No germination
2 5/6	,,	Germination taking place in succession
3 1/6	,,	Germination increased, encysted gametes numerous
23.00	Germination by 60 per cent., gametes mostly encysted.	Germination by 90 per cent., gametes mostly encysted
27.00	Germination still taking place	Almost all germinated

Thus the germination takes place, though much delayed, at 8–9°C. It is shown that the rate of germination in individual gametangia extends in a wider range than at higher temperatures.

In other drops exposed to 6°–7°C. the germination was found to take place, but in those exposed to 5°C. no gamete was liberated.

3. Some hanging drops were exposed to 25°–26°C. and the others to 19°–20°C. as a control, and the feature of germination was noted at intervals.

2 hours and 40 minutes after: No one germinated; in the control drops cloudy gametes appeared, being mostly at rest.

3 hours and 30 minutes after: In each drop one or two gametangia germinated, the gametes being in sluggish movement; in the control gametes increased progressively.

5 hours and 30 minutes after: No further germination; in the control about 50 per cent. of gametangia germinated.

The drops were immediately exposed to a temperature of 19°C. 50 minutes later, the process of the gamete formation was clearly

visible in the gametangia by 50 per cent., and in further 10 minutes the gametes were liberated in the drop in great abundance.

Thus we see that the germination was greatly hindered at 25°-26°C.

3. Relation to Medium

In ordinary experiments of germination tap water was used as the medium. In comparison other media were tested to see what influence they exerted upon germination.

In distilled water the germination begins a little earlier than in tap water. The liberated gametes are generally very active and are often provided with 2–3 oil-drops instead of a single one. It gives an appearance as if absorption of water by the gametangia proceeds more rapidly, on account of which they rupture a little earlier than usual. In other respects the distilled water shows no difference from the tap water in relation to the germination of gametangia. In rain water and well water the process of germination is observed to be the same as in the tap water.

Extracts of soil from the ground where the affected host grows were tested at various concentrations. In a concentrated extract the germination did not take place, but when it was diluted with water the gametes were liberated profusely. They were in some cases sluggish, soon settling down, while in others they were as active as in tap water. It is evident that the soil water acts, at a definite concentration of dissolved substances, with inhibitory effect upon the germination of gametangia.

The water exudated from water-pores of the host plant was found to check the germination. We see that it does not participate in the germination of gametangia and the infection of the host.

In the 1.5–2 per cent. solution of cane sugar gametangia proceeded to form the gametes inside but were not able to dehisce, or else the liberated gametes were very sluggish and soon settled down.

I did not go further into this subject, as it is not the chief aim in the present work. So far as observed, it appears that the germination of gametangia is sensitively influenced by the presence of certain soluble substances in the medium. Distilled or comparatively pure and fresh water, such as tap, well, and rain water, is the most favourable medium for the germination of gametangia and for the liberated gametes.

4. Viability

Gametangia dispersed on the surface of the host around the dehisced sorus tend to lose the viability from drying sooner than those remaining within that sorus. Dead gametangia fade in colour. As long as a fresh appearance and orange colour are retained, the gametangia are mostly able to germinate.

The longevity of viability depends upon temperature. In summer the gametangia lying on diseased leaves persist in their viability for a week or two, but in spring and autumn they remain alive still longer. Some of the tests on the longevity of gametangia at different seasons are recorded below. Gametangia upon the diseased leaves were kept in a room under a bell jar, and their germination was tested at an optimal temperature (20°C.).

1 (May, 1925). The 13-days old gametangia were fresh in appearance, being yet orange in colour. The germination went on quite normally.

2 (July, 1925). The 13-days old gametangia remaining in dehisced sori showed a high percentage of germination. The 50-days old ones inside the sori were tested at the same time. Though they appeared fresh and alive, germination did not take place except in a few of them.

3 (August, 1926). Gametangia 45-days old were found mostly dead. At the bottom of sori there remained some that seemed fresh in appearance, but they could not germinate.

4. Longevity of gametangia lying at low temperatures was tested.

(a) A pot plant inoculated on October 30, 1925 produced dehiscing sori on November 30. On December 27, the gametangia nearly a month old have shown the normal course of germination. The affected leaves were then preserved in a room with a temperature ranging from 5° to 10°C., and at intervals the germination was tested at about 20°C. On the 40th, 58th, 72nd, and 83rd day most of the gametangia so tested have germinated. On the 100th day the leaves were attacked by mould, but the gametangia remained still healthy and could germinate. Further longevity was not examined.

(b) A pot plant was inoculated on the middle of October of 1927. The pot was kept in a room. Owing to a low temperature the development of both the host and the fungus proceeded very slowly and the

summer gametangia came to maturity at the end of November. Under a bell jar the affected leaves remained alive in the room throughout the winter till the middle of April of the next year. During the winter the room temperature fluctuated between 5°C. (at night) and 17°C. (at day). The gametangia spread over the surface of the leaves were dead during that interval, but in the sori some number remained appearing alive. On applying pieces of the affected leaves bearing these gametangia to other healthy pot plants infection was obtained and after two weeks diseased patches became distinctly recognizable. In this experiment it was shown that at low temperatures the gametangia were viable for nearly 5 months.

(c) On January 30, 1925 an affected rosette of *Oenothera biennis* was found outdoors under a shelter, being protected from any injury by frost. In some of the diseased leaves the summer gametangia remained in a fresh condition at the bottom of the sori. Probably they had been produced at the late autumn (November) of the preceding year. Bringing them in water at 20°C. germination took place normally. Thus the gametangia had been viable in the field over two months during winter.

From the above we see that the viability of gametangia is influenced by the temperature. At lower temperatures it can be retained safely for five months.

5. The Gamete

a. Description

The gamete (hitherto called the zoospore) of *Synchytrium* is often described as being spherical. In the present species Schröter (cf. Tobler, 1913) also gives it to be spherical. So far as observed by me, the form is not constant. In an actively swimming state it is typically pear-shaped, while during sluggish movement a shorter and broader, or spherical form is assumed. When the gamete is for a while stationary, as is often observed, it is spherical, though the original pear-shaped form is soon resumed on swimming. When the gametangium is submitted to germination at high temperatures, masses of immovable spherical gametes are often released, but on coming to motion each gamete assumes a pear-shaped form. Therefore, the gamete of *S. fulgens* is said to be typically pear-shaped. In rare cases some gametangia produce gametes acquiring a form with broader apex during swimming.

The typical gamete is provided with a single orange oil-drop behind a central nucleus. Two or more drops of unequal sizes are usually found when the gamete is produced at supraoptimal temperatures or under other unfavourable external conditions. According to the size of gametangia the number of gametes derived from each gametangium varies from 150 to 300, but in the majority about 200 are produced.

b. Encystment and the Fate of Cilia

After swimming for a certain length of time, the gametes come to rest, sometimes attaching to the substratum, but mostly floating on the surface of water. On coming to rest they acquire a spherical form and tend to increase in size, perhaps by absorbing water. Vacuoles, increasing in number and size, make the cytoplasm reticulate. The outline of the body is becoming distinct; the presence of the membrane can be clearly recognized when the cytoplasm shrinks on addition of a weak alcoholic solution of gentian violet to the medium. It is probably a plasmic membrane.

Later, the cytoplasm looks as if more condensed and appears more refractive. At lower temperatures this structure is retained for more than 24 hours.

Immediately after rest the cilium can be recognized as being stretched or in a gentle swinging movement. When encystment ensues, it disappears from sight. In the living material an exact observation on the fate of the cilium is almost impossible, but by staining with a weak alcoholic solution of gentian violet, the cilium is clearly visible. While the cilia are not found on the encysted or rounded gametes, there appear numerous stained fibres arranged densely along the margin of the drop of water used as a medium for germination. As they usually run parallel closely together, individual fibres are often not distinct, in particular when the drop contains innumerable gametes already encysted. In the drop with a small number of the encysted gametes the fibres run loosely, sometimes crossing one another or scattering here and there. In this case the length of the fibres can be easily measured. It is approximately the same as that of cilia.

Broadly speaking, the number of fibres present in the drop of water accords with the number of the encysted gametes: while the gametes are yet all swimming, no fibre is visible in the drop.

When Flemming's solution is added to the drop previous to staining by gentian violet, the fibres assume a beaded appearance, just as

manifested by the cilia yet attached to the gametes. Therefore, it is evident that the fibres represent the cilia dropped from the encysting gametes. These detached cilia persist till the next day, or still longer, without undergoing disintegration.

In several kinds of swarm cells hitherto investigated the fate of the cilia is not the same in all; in some organisms the cilia are intruded into the body, while in others they are dropped on coming to rest or to encyst. In *Olpidium Viciae* (KUSANO, 1912) intrusion of the cilia is observed in disintegrating planogametes, but in encysting individuals the cilia are found intact until encystment begins, their sudden disappearance being due to dropping from the body. In disorganizing gametes of *Synchytrium fulgens* intrusion of the cilia may probably occur as in *Olpidium*, but the fate of the cilia is otherwise in encysting individuals.

The encysted gametes, whether floating on the surface of water or deposited at the bottom of the medium, become afterwards unwetted and assume a refractive appearance. The significance of this change is not known.

High temperature, presence of certain substances in the medium, or deficiency of oxygen acts upon the gametes as preventive of encystment; settling down in the medium they round up, are swollen, become more hyaline, and at length are disintegrated, leaving oil-drops behind.

The presence of the blephaloplast may be ascertained in the living material after encystment. It is recognized distinctly when stained with acetic methyl green or alcoholic gentian violet. By staining it is visible even at a stage previous to encystment. In the living state it lies as a hyaline refractive spot along the membrane of the body. As long as the periphery of the encysted body appears hyaline, the blephaloplast is clearly detected, but later its presence is obscured on account of the reticulate structure of the plasmic content.

c. ABNORMAL FORMS

At or below 20°C. the gametangia liberate gametes of a typical form, but above that temperature the derived gametes present certain abnormalities. As representing a form slightly deviated from the normal, they contain two orange drops instead of one. At 22°–23°C. both one- and two-dropped gametes may be liberated from the same gametangium, and at higher temperatures, for instance, 24°–25°C., pre-

dominance of two-dropped ones becomes apparent. In behaviour such gametes exhibit no difference from the typical ones.

When the temperature is further increased, for instance, to 28°C., multiciliate and many-dropped gametes of varying sizes are produced. They represent compound gametes. They are released from the gametangium in an immovable state, but after a while the cilia begin to move, enabling the gametes to swim. The compound gametes are generally sluggish in movement, soon come to rest, and are disorganized.

At high temperatures it frequently happens that the gametangium ruptures while the segmentation of the content into gametes is still in progress, and consequently a number of large segments are released remaining immovable. Each segment is not provided with cilia, so that it represents a plasmic mass in which the process of forming the gamete advances but very little.

Compound gametes can be produced more frequently when the gametangia are brought at first to 20°C. for one hour to one hour and twenty minutes and then to 28°–30°C. Being at these higher temperatures for 40 minutes some of the gametangia germinate. It may be noted that the interval of time required for germination is in this case far shorter than that in gametangia exposed from the first to the higher temperatures, but nearly the same as when the gametangia are exposed to 20°C. throughout. The gametes are mostly abnormal; the two-dropped forms are released, being first immovable but after a while swimming away, and the compound forms of varying sizes are usually found among them.

In altering the temperature as just mentioned the formation of the gamete may appear to proceed as follows. While the gametangium is at 20°C., the condensation of the orange oily substance and the segmentation of the cytoplasm are proceeding at the usual rate, but being brought to the higher temperature their progress is more or less retarded, though the dehiscing process of the gametangium is advancing unarrested. The result is that the gametangium ruptures while the formation of the gamete is not yet completed.

From the above experiment we distinguish three essential physiological processes during germination, viz.—(1) the condensation of the oily substance, (2) the segmentation of the cytoplasm into gametes, and (3) the production of osmotic substances in the gametangium. At an optimal temperature (20°C.) they proceed with the same pace, but higher temperatures give disturbance to the balanced condition of their progress, retarding the former two processes.

d. The Swarm Period

The duration of swimming presents more or less variations according to temperatures, to which the gamete is exposed. In repeated experiments at an optimal temperature (20°C.) a large number of gametes are going to rest, on the average, at 30-40 minutes after a pronounced germination occurs. At temperatures below or exceeding 20°C. the swimming activity is retained longer, except at so high a temperature as to produce abnormal gametes. The general feature of the relation of the swarm period to temperature may be presented in the experiments to be given below.

1. Gametes were liberated in several hanging drops at 18°–19°C. After 3 hours they were found in great abundance and a large percentage had already gone to rest. Some of the drops were then exposed to 26°C. Examined one hour later, by far a greater number of the gametes were yet swimming actively, while those in the drop which remained at the original temperature attained mostly the resting stage.

The influence of a reversed alteration of temperature was tested. Some drops were first exposed to 26°C. for 3 hours. Germination was retarded but some gametangia seemed to have germinated just at 3 hours. One set of the drops was then brought to 20°–21°C. Examined one hour later, by far a greater number of the gametes had been at rest than in the other set of drops which remained at 26°C.

2. Gametangia were submitted to germination at 19°–20°C. After one hour and a half, while the gametes were just being densely liberated, one set of the drops were exposed to 26°C. In this set the period of rest was attained one hour later, while in the set remaining at the original temperature the resting gametes were found to be increasing already during that interval.

3. Three sets of hanging drops were prepared. The first set was exposed to 20°C. Germination began at one hour and 20 minutes later. In further 30 minutes the gametes were going to rest.

The second set was exposed to 28°–29°C. After 5 hours germination was not observed.

The third set, after being exposed for 3 hours to 28°–29°C., was transferred to a place at 20°C. One hour and a half later, the gametes were being liberated in abundance. The drops were immediately exposed again to 28°–29°C. Almost all gametes were swimming for more than one hour.

4. Gametangia were first submitted to germination at 22°C. After one hour and a half they began to germinate. Some of the drops were then exposed to 6°–8°C. Observed one hour and a half later, the gametes were yet swimming, except a few already at rest. Thus the swarm period was prolonged at low temperatures.

From the experiments given above we conclude that the swarm period of the gametes is shortest at an optimal temperature (20°C.) for germination and a prolongation of $\frac{1}{2}$–1 hour is observed at extra-optimal temperatures.

The conclusion thus arrived at is based on the observations made on the group of gametes derived from different gametangia at different times. Therefore, an estimation of the swarm period is evidently not accurate, since the time of liberation was not ascertained in each gamete. In the hope of knowing more exactly the length of the swarm period a single gametangium was brought to germination in each fine drop of water upon a cover glass. By this means we could know the time of germination in each gametangium and in consequence the time when swimming started in all sister gametes. Upon examination of numerous gametangia thus treated a remarkable result was obtained. The swarm period presents a considerably wide range of variation, according to individual gametangia and also to individual gametes from a single gametangium.

So far as observed at various temperatures (7°–20°C.), the range of the swarm period of sister gametes extends in an extreme case from a few minutes to 30 hours after liberation. During this interval of time the relative number of individuals coming to rest at successive periods are very variable according to different gametangia examined. A large number attain the rest period within one hour, while the remaining continue all their swimming movement far longer; or only a few come to rest within 10 minutes, while no one comes to rest during further several hours. Moreover, beginning at a period of a few to 10 minutes after liberation, the sister gametes come to rest in one case all in a rapid succession within further 30 minutes. In the other case they come to rest periodically; some number come to rest within 10 minutes; in subsequent 20–30 minutes no one comes to rest; and in the next 10 minutes a large number become sedentary in succession; but after this period a long duration passes without any increment of sedentary gametes; and finally the period of rest is attained by all swimming ones.

Of several gametangia submitted to germination in different drops on the same cover glass, some produce the gametes coming early to

rest, while others produce those which swim for a longer time, ranging from 1 to 30 hours.

On this account we may for the sake of convenience distinguish short-swimming and long-swimming types of the gametangium, according to the length of the swarm period of the gametes which they produce. In the former type a great majority of gametes come to rest in 2 hours, while the latter type comprises those gametangia producing gametes, most of which maintain their activity beyond 2 hours after liberation (refer to the experiments in Sec. V).

From this fact we learn that to determine the effect of temperature upon the swarm period of each gamete is impossible. Further we see that the conclusion given above on the relation between temperature and the swarm period should be understood as follows. In a mass of gametangia taken from a definite diseased patch a certain number of gametangia of the short-swimming type were contained, and in another mass from the same patch the gametangia of the same type might have existed in nearly the same proportion. The gametes derived from the gametangia of this type came in both masses to rest at an earlier period of observation than those from the gametangia of the long-swimming coexistant type. The effect of temperature ascertained in the previous experiments has been exhibited by these gametes, those in one mass at a certain temperature coming earlier or later to rest than those in the mass at another temperature.

The swarm period has a great influence upon the formation of the zygote and it will again be dealt with in a later section.

e. Phototaxis

While observing the gametes in a hanging drop of water I was impressed with their local assemblage at the brightest side of the drop. By rotating the cover glass carrying the drop the gametes left the original place to come within 5–10 minutes to a new brightest side.

A similar phenomenon was macroscopically observed in water of a glass vessel, in which a vast number of gametes were found in suspension. Standing overnight near a window and observing in the early morning, an orange precipitate was visible on the brightest side at the bottom of the vessel, opposite to the window. Submitting gametangia to germination in the morning and observing after a few hours, an orange cloud was found along the wall of the vessel on the side facing the window.

I did not enter into detail on this reaction of the gametes. Only it may be added here that the reaction to light is influenced by temperature. At an optimal temperature (20°C.) the reaction is most sensitive, but at a temperature exceeding that the sensibility is becoming feeble. For instance, bringing the hanging drop from 20° to 27°C. the local assemblage of the gametes does not take place.

IV. The Germination of the Resting Cell

I. Formation of the Gametangium Sorus

Until germination begins the content of the resting cell appears translucent on transmitted light and white on reflected light. Large, angular globules, densely arranging in the cavity of the cell, show in fresh as well as stained materials a nuclear structure, being studded with chromatin-like granules on their periphery. They are plasmic inclusions, perhaps a kind of reserve materials, and on germination they undergo dissolution, whereas the content of the cell becomes densely granular, more opaque than before, and also yellowish.

Following this change a small, thin-walled, hyaline sac is protruded from the inside of the cell to the surface through a pore made on its wall (Fig. 2). The sac grows larger and larger. At first it appears vacant, but soon the plasmic content of the cell flows out into it. As this migration advances, a clear widening space is visible in the cavity of the cell at the side opposite to that where the sac appears. When about half an amount of the content is migrated into the sac, the large nucleus begins to pass through the small pore by constriction.

An enlargement of the sac continues till the cell is exhausted of its plasmic content. The sac is quite spherical, strictly corresponding in volume to that of the cavity of the resting cell.(1)

The content of the sac is finely granular and very dense. On reflected light it appears milky white, though on transmitted light a somewhat opaque matrix appears yellowish orange. Probably, the content is in an emulsion state. The colouring matter and fine granules seem to have been generated from the angular globules by dissolu-

(1) It may be noticed that in the external appearance the resting cell at this state is exactly similar to that of *S. endobioticum*, on which a zoosporangium of *Phlyctochytrium synchytrii* is developed (KÖHLER, 1924, b).

tion. Details will be given on studying microtome sections. So far as observed in fixed materials *in toto* the following facts may be mentioned.

The substance which renders the plasmic content of the sac opaque is stained deeply black with FLEMMING's fixing solution. Angular globules existent before germination are not stained homogeneously; their matrix is less stained, while the granules upon it become black. Approaching germination, however, the globules stain homogeneously black, assuming a drop-like appearance. The period and degree of blackening with FLEMMING's solution coincide with those of an opaque appearance during the progress of germination. It appears to show that the reserve material (angular globules) is changing into oily substances.

The pore on the wall, through which the sac bulges out, is comparatively small, measuring approximately 4.5 μ in diameter with double contour lines on surface view.

After the content of the resting cell is wholly migrated into the sac, the pore is closed with a plug made of a hyaline substance (Fig. 1). At this time the wall of the sac is, at the portion surrounding the pore, more or less thickened inwardly into the shape of a convex lens. The plug projects into the cavity of the resting cell as a conical, spherical, or club-shaped process (Fig. 1). A careful examination reveals its material continuity with the endospore and the wall of the sac. As to whether these three portions are composed of the same substance we cannot state yet with certainty.

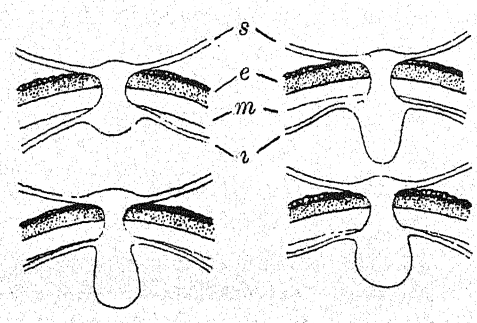


Fig. 1. Various forms of the plug in the germinated resting cell. *s*, wall of the winter gametangium sorus; *e*, epispore of the resting cell; *m*, mesospore; *i*, endospore. (×800)

After the formation of the plug, the opaque substances diminish, and the sac appears transparent and distinctly orange-coloured. Now cleavages appear in its content (Fig. 3). They are preceded by an appearance of hyaline lines in the coloured matrix, representing primordial membranes made of substances secreted from the plasm. These membranes divide the whole plasmic mass of the sac into a number of polyhedral segments, each developing into a gametangium. Perhaps owing to a swelling action of the segments, furrows are produced inwardly along the limiting membranes, so that

each segment is separated from each other in a round form. The wall of the sac, now the wall of the gametangium sorus, is caused to rupture by an internal pressure exerted by swollen gametangia, and thus mature gametangia are set free in the surrounding medium. Often some sori remain for a long time undehisced.

As already described, the resting cells are far smaller than the summer sori developed simultaneously on the same diseased spot. In consequence, the winter sori derived from them are correspondingly smaller. The size of gametangia, though more or less varied, does not on the average differ much from that of the summer form. It shows that the winter sorus contains a less number of gametangia as compared with the summer sorus. In a medium-sized sorus 15–16 and in a larger one about 21 gametangia may be found, while in the sorus originated from a smaller resting cell only 2–3 are present. Perhaps an unigametangium sorus may possibly be produced, though we are not as yet able to find it.

2. Temperature and the Rate of Germination

The rate of germination of the resting cell presents a great individual variation. When a mass of the cells is submitted to germination at a certain temperature, only a few come first to germinate. Day after day the germination is occurring in other cells and after a longer or shorter duration a majority succeed in germination, leaving, however, some number ungerminated for an indefinite length of time. As regards the rate and duration of germination the temperature seems to be concerned in a great measure. At low temperatures germination occurs in a long duration, but at certain higher temperatures most cells begin to germinate within a short duration. The general features will be found in the table given below (Table III). The material was collected in early autumn and being freed of decayed tissues of the host had been preserved for 2–6 months under slightly moist conditions until used for the experiment.

When the resting cells are exposed to a high temperature with a daily fluctuation of 22°–30°C., no one comes to germination within a few days. Lowering then the temperature to 19°–7°C. a few germinate after 2 days and more than 50 per cent. after 12 days. It shows that the high temperature checks the germination.

In order to test the action of lower temperatures a mass of resting cells, being immersed in water, was kept during winter a few centi-

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meters deep underground. For about the first 3 weeks the ground remained frozen throughout day and night, and the temperature did not exceed 5°C. The resting cells remained dormant during the interval. Afterwards the temperature was getting higher and at day time it exceeded 10°C. Germination could be observed after a month.

TABLE III

Showing the rate of germination of resting cells at different temperatures. The figures show the number of the days after sowing

Daily fluctuation of temperature (C.)	Appearance of the sac	Dehiscence of the sorus	Germination over 50 per cent.
18°–26°	3	4	5
17°–20°	2	5	4
13°–14°	4	10	7
7°–19°	6	13	14
6°– 8°	11	27	22

From the above experiments it will be seen that an optimal temperature lies near 20°C., at which germination begins on 2–3 days, leading more than 50 per cent. to germination in 4–5 days. Below or above that germination is delayed and takes place in a long interval of time. At about 30°C. and 5°C. germination is prevented.

The time taken by each consecutive process involved in germination, from the beginning of the sac formation to the completion of the gametangium sorus, is also correlated with temperature. For instance, at a range of 13°–17°C., the time required by each process is as follows:

a. 5–6 hours for the completion of the sac. In one of the cells watched under the microscope the formation of the sac proceeds as in the accompanying figure (Fig. 2).

b. 2–3 days from the completion of the sac to the beginning of segmentation.

c. 5 hours for the completion of segmentation.

d. 10 hours from the completion of segmentation to the maturation of gametangia, that is, to the separation of the segments into rounded gametangia.

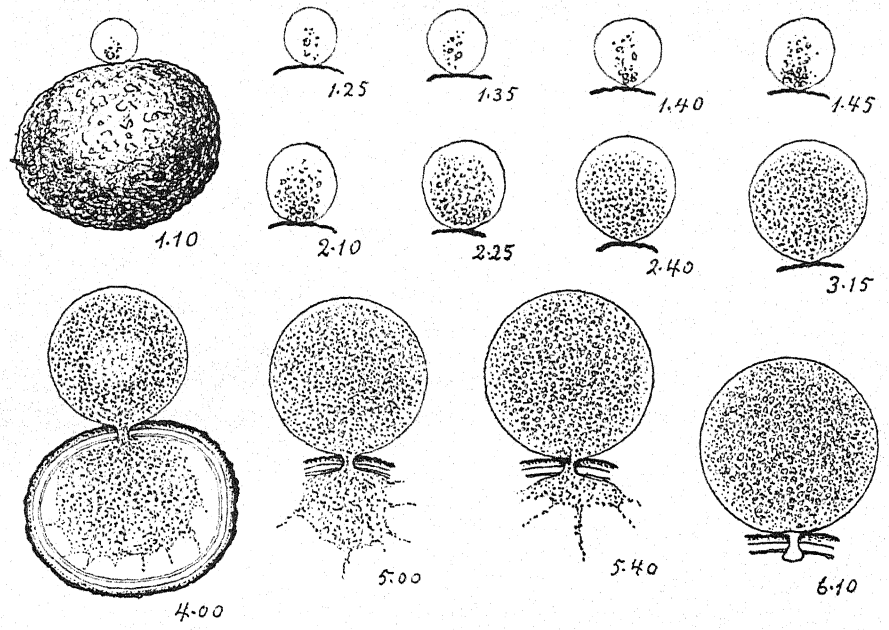


Fig. 2. The course of germination of a resting cell at about 17°C. (Numerals denote the time of observation in p.m.) (×400)

e. 4 hours (at an earliest rate) for the dehiscence of the gametangium sorus. Generally the sorus may remain undehisced for a few hours or more.

Altogether 3–4 days are required from the beginning of germination to the maturation of the gametangium.

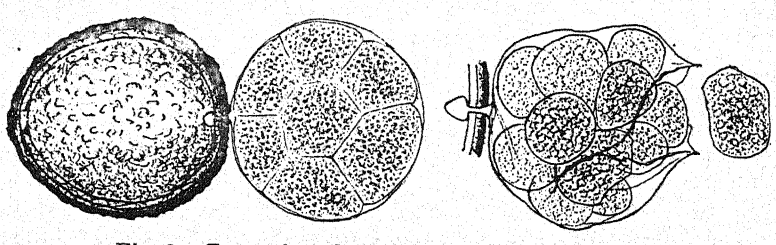


Fig. 3. Formation of the gametangium sorus from the resting cell. (×400)

It may be noticed that the duration between the completion of the sac and the beginning of segmentation is comparatively long. As may be understood from what has already been mentioned, there may occur during this interval chemical changes in the reserve material and the repeated nuclear divisions (about 7 times).

Exposed to a wider range of temperature (falling at night to 10° and rising at day to 18°C.), the progress of the germination process is somewhat slow, the lower temperature being responsible; for the formation of the sac 4–7 days and for segmentation 1–3 days are required.

When the germination is going on at nearly a constant temperature, say 19°–20°C., the process is much accelerated. In a few resting cells mounted on a slide the germination was observed to proceed as given below (Table IV).

TABLE IV

Showing the rate of germination of the resting cell at 19°–20°C.

No. of cell	Day after sowing									
	1	2	3	4	5	6	7	8	9	10
I	—	—	Sac	Sac	Sorus (dehis.)					
II	—	—	Sac	Sac	Sac	Sorus (dehis.)				
III	—	—	—	—	Sac	Sac	Sorus (dehis.)			
IV	—	—	—	—	Sac	Sac	Sorus (dehis.)			
V	—	—	—	—	—	—	Sac	Sac	Sorus	Dehis.

In general the sac remains unsegmented for 2 days, and in the next day segmentation takes place and the sorus attains the maturation period.

It may be concluded that the optimal temperature for the formation of the winter gametangium sori from the resting cells is about 20°C., just as that for the germination of the summer gametangia.

In the resting cell of *S. endobioticum* the optimal temperature for its germination lies, according to CURTIS (1921), between 12° and 14°C. and according to ESMARCH (1928, p. 95) between 19° and 20°C. It appears that both fungi do not differ in this respect.

3. Effect of High Temperatures on the Viability

It has been shown in the foregoing that a temperature as high as 30°C. acts upon the resting cell as inhibiting its germination. However, the viability is not lost in this case; after being exposed to that temperature for 4 days and then transferring to lower temperatures (for instance, 17° or 20°C.) germination takes place two days later, and its percentage is increased day after day, the result being nearly the same as when the cells are exposed from the beginning to the optimal temperature (20°C.).

To test the action of still higher temperatures some experiments have been carried out.

1. Air-dried cells, after lying in water for a few hours, were exposed to 50°C. for 24 hours, and afterwards to 20°C. for 3 days. No germination took place. Hereafter the cells stood in a room with a temperature ranging from 13° to 18°C. For the control another mass of cells was exposed from the beginning to the room temperature.

10 days after: No germination; the control cells germinated by 50 per cent.

17 days after: A few had germinated; percentage of germination increased in the control.

37 days after: A great majority germinated in nearly the same percentage as in the control.

Thus high temperatures delay the germination of the resting cells. As to whether the said temperatures exert an inhibitory action upon germination or kill some of the cells that are able to germinate at an early rate, a decisive conclusion cannot be given. We consider it possible that the later germinating cells may be more resistant to the action of high temperatures than those germinating early and can retain their viability.

2. A mass of the cells was exposed after being soaked with water to 60°C. for one hour and then left in a room where the temperature varied from 16° to 20°C.

9 days after: No germination; in the control the germination took place by 80 per cent,

18 days after: No germination; percentage of the germination increased in the control.

Examined on succeeding days, no germination was ascertained at all; certainly, the cells had all been killed.

GLYNNE (1926), studying the resistance of the resting cells in *Synchytrium endobioticum* to heat, obtained a similar result as to the thermal death point. In wet materials 1–8 hours exposure to 60°C. was sufficient to kill the cells. WEISS and BRIERLEY (1928) accord with her in this respect. Extensive tests were not made in our fungus, but it may be said that the resting cell of both fungi shows a similar degree of resistance towards heat.

4. Dormancy and Vitality

In the experiments already given it has been shown that the resting cells preserved for some months can germinate within a few days when submitted to germination under favourable conditions. In the following experiments an attempt has been made to determine whether they require a dormant period of certain lengths, or that they demand exposure to dryness or cold during the dormant period.

1. On September 3, 1925 the resting cells were collected from the diseased leaves and stems already dead and more or less decayed. The cells appeared to have been fully matured, and had been exposed to alternately wet and dry weather. By crushing gently the diseased host tissue between the fingers the cells were isolated in a pulverous form. After being washed repeatedly, they were immersed into water at a temperature of 18°–19°C.

Examined after 3 weeks, a few cells were found already germinated, and after 30 days there were found numerous gametangia mostly freed of the sori. 10 days later, the germinated cells amounted to 80–90 per cent.

From this experiment we see that the resting cell can germinate without wintering.

2. On October 5, 1925 the isolated cells collected at different seasons were submitted to germination.

(a) The cells stood in the field on dead stems and leaves till the day preceding the experiment. They had been exposed to low and high temperatures under alternately wet and dry conditions.

(b) The cells were collected on August 10 and preserved at 18°–19°C. in a more or less moist air.

(c) The cells were collected on September 2 and preserved for two weeks in an ice chamber with a temperature of 5°–8°C. and afterwards in a laboratory room with a temperature ranging from 16° to 18°C.

In all sets of materials the germination went on similarly; some cells had already germinated after 23 days, and the germinated cells amounted to about 30% after 38 days and 50–80% after 52 days.

3. At the end of August, 1925 the mature cells freed of the dead tissue of the host were collected, and one part was stored air-dried and the other underground till November. They were then brought to germination at a temperature rising to 18°C. at day and falling to 10°C. at night. In the air-dried material the germination began after 5 days, while in the underground material it took place 2 days earlier. In both the same percentage of germination was attained after 3 weeks (50%). In this experiment a little delay of germination in the air-dried material seems to be due to the fact that a certain time is required to absorb water preparatory to germination.

4. Pieces of fresh leaves bearing the recently matured cells were immersed in water and other pieces of the same leaves were alternately air-dried and wetted each week during about 3 months. Then both materials were brought to germination in fresh water at a room temperature (5°–17°C.). Germination began in the undried material after 34 days, while the material repeatedly dried began to germinate 10 days later. Observed after 54 days, the percentage of germination was the same in both materials. In this experiment it is shown that the mature resting cells are able to germinate without a notable period of rest.

Regarding the rate of germination it must be taken into account whether the cells remain inclosed in the host-cell or are freed therefrom. The freed cells germinate earlier than those enveloped with the brown dead wall of the host-cell. For instance, at a room temperature in May and June the germination begins in 5–10 days in the freed cells but in one month or more in those lying in the host-cell. How the delay of germination is brought about is not yet investigated. We may suppose that the wall of the host-cell, together with its disorganized content, acts as preventive to the absorption of water by the resting cell. In water its putrefaction takes some weeks, being accelerated by high temperatures. The germination seems to commence after this obstacle is more or less decomposed.

From the experiments so far carried out we may draw the following conclusion:

1. The rate of germination in the resting cell is essentially the same whether exposed to a lower temperature or not, and whether kept dry or moist.

2. The mature cells whether lying in the living or dead host tissue, or stored air-dried for more than 3 months show nearly the same percentage of germination during the same interval of time.

3. Germination begins a little earlier in the cells preserved under a moist condition than those stored air-dried.

4. Germination can take place without a notable period of rest.

5. The resting cells inclosed in the host-cell is delayed in germination.

So far as observed, we see that *Synchytrium fulgens* and *S. endobioticum* behave similarly with respect to the course of germination of the resting cell. In *S. endobioticum* WEISS (1925) succeeded in causing infection in December with the resting cell collected in September of the same year and stored indoors. KÖHLER (1924) has shown that freezing has no influence upon the rate of germination. Regarding the rate of germination the results obtained by several authors are not in accordance. Some authors observed the gametes in 3–5 days, some in 20 days, while others in 10 weeks. There is a great probability that such variance is caused by different external and internal conditions of the examined resting cell, as ascertained in *S. fulgens*.

An investigation into the longevity of viability has been made, and is now in progress, with the materials collected in different years and stored under different conditions.

The resting cells freed of, or inclosed in, the host-cells were stored air-dried or in soil. Severely infested soil was also stored air-dried in a room. After just one year these materials were tested for germination. In all of them germination was observed within the same interval of time as required by the cells which are a few months old.

The air-dried materials collected in the year of 1925 were tested in 1927, i.e., after two years. Germination took place normally.

The oldest material available was furnished by the herbarium specimen of the diseased host plant which was collected in October, 1922. Some of the leaves already dead at that time had borne numerous resting cells which might be considered by estimation to have matured during August. After three years (December, 1925) pieces of this material were brought into water and left in a room at a temperature ranging from 5° to 18°C. Examined after 2–3 days the whole aspect of the resting cells revealed no difference from that presented by those cells recently matured. After 35 days yellow sacs appeared on the surface of the decayed host, and several stages of germination, from the beginning of segmentation to the dehiscence of the sorus, could be

observed. After 43 days a large percentage had germinated. The retention of vitality for three years was thus clearly demonstrated.

To test further longevity the same material was submitted to germination on June, 1927, after 5 years. First on coming to the 13th day two or three yellow sacs appeared among a mass of the resting cell. They increased in number day after day and a number of gametangium sori were produced.

On May, 1928, after nearly 6 years, the germination test was made. After a month some resting cells came to germination.

Lastly, on May, 1929, pieces of the host bearing the resting cells were kept in water at a room temperature. After a month, when the host tissue was rotten, the germination took place in some cells and mature gametangium sori were obtained.

We conclude therefore that the viability of the air-dried resting cell may be retained for 7 years. To determine the limit of longevity we need further examinations.

The next point to be considered is the longevity in the resting cells which remain in water. In the germination experiment it has been ascertained that most of them germinate during an interval of a month or two under favourable conditions. On examining an old culture which stood stationary over 10 months I was struck with the fact that the germination was still occurring. It showed that most cells germinated in an early part of the period, but there some number remained dormant for a longer period and came to germination at times.

The oldest culture now in possession has just passed over three years. The material collected in 1922 and stored air-dried was brought into water at the end of 1925. When examined after two years (August, 1927), the culture was found contaminated by unicellular algae. Though most of the resting cells were already empty, yet there remained a few per cent. appearing like those recently matured. Besides, there was found some number of young yellow sacs whose content was not yet segmented. Furthermore, when a mass of the cells was mounted on a slide, germination occurred in several cells during a subsequent week or two.

After three years (August, 1928) intact dormant cells became exceedingly fewer. Still it was possible to observe the germination taking place in some of them. Lastly after 3 1/2 years (June, 1929) new sori appeared when the material taken from the stock had been spread over a filter paper and kept sufficiently wetted for some weeks.

In the resting cell of *S. endobioticum* the longevity of viability has received much attention of several authors. SCHAFFNIT and VOSS (1918) reported that in soil once infested with the parasite and left uncultivated the capacity for causing infection is retained for 9 years. COLLINS (1921) stated that the viability is retained for 4 years in artificially stored materials. Recently ESMARCH (1928) found persistency for $3^1/_2$ years in water. WEISS and BRIERLEY (1928) showed that nearly 6-years old gametangia in dry wart materials are still viable. As mentioned above, the resting cell of *S. fulgens* is viable at least for 7 years in a dry state and may remain dormant in water during $3\ ^1/_2$ years. It is interesting that this record accords with that given in *S. endobioticum*.

The variation of the dormant period in water is of special interest. In this respect the resting cells closely resemble some phanerogamic seeds, in which germination is often delayed for many years. Deficiency of oxygen needs not account for the delay in this case. In the seeds lying underground structural variations of the seed coat may bring about variations in the rate of water absorption. I am inclined to the view that a similar thing may hold true with the dormancy of the resting cells in water. As will be given later in detail, the formation of the epispore is controlled by the condition of the host-cell and its permeability to water may not be constant throughout all individuals of the resting cell. Further, the resting cell usually remains inclosed in the disintegrated content of the host-cell and in its thickened and durable wall. These envelopes appear to exert a certain influence upon the absorption of water by the resting cell. In other words, the delay of germination is essentially correlated with the delay of absorption of water.

The fact obtained while making paraffin cakes with a mass of the resting cells submitted to germination may give support for this view. Among still ungerminated cells some are not or are less stained with osmic acid contained in the fixing agent and also are not penetrated by paraffin; perhaps they have remained incapable of absorbing water.

In the case of *S. endobioticum* ESMARCH (1928) explains the dormant period in connection with the physiological condition of the protoplast, which can be expressed by the "after-ripening." He states (p. 36), "Das Wesen der Nachreifung besteht darin, dass die Anlagen der Zoosporen (Primordien) sich in vollentwickelte Zoosporen umwandeln." As will be reported elsewhere, I can ascertain in our fungus the presence of a primordium-like body, but it is in fact a reserve globule.

Therefore, I greatly hesitate to accept his explanation of the same phenomenon in our fungus.

5. The Germination of the Gametangium and its Condition

After the process of germination in the resting cells advanced progressively till the mature gametangia were freed of the sori, it was expected that the germination of gametangia would immediately follow. Yet, frequent examinations of the culture during one or two weeks failed to confirm their germination. Examining the culture after a month, the successive germination of the resting cells had produced a considerable number of gametangia, all appearing healthy but remaining ungerminated. A similar case was experienced by RYTZ (1907) in other members of *Synchytrium*; he succeeded in several species in obtaining gametangia from the resting cells, but his attempt to observe their germination was quite in vain. It appears very curious that the condition favourable for the formation of gametangia from the resting cells does not allow the gametangia to germinate.

As a first trial for elucidating the matter concerned, a few gametangia were taken by means of a capillary pipette from a stock culture preserved for more than a month and were mounted on hanging drops of fresh water. To my astonishment the germination took place in a few hours and numerous gametes were liberated in the medium. The process of germination was quite the same as already described in the summer gametangium. The trial was repeated several times and always the same result was brought about.

Next fresh water was added to the stock culture. The effect was distinctly manifested. Examined on the next day, liberation of a vast number of gametes was confirmed.

To afford further evidence for this fact the following experiment may be mentioned.

From an old stock culture masses of the gametangia were transferred to three hanging drops of water on March 20, 1926. On the next day the gametangia in all drops have germinated by 50 per cent. The drops stood until March 28, a week long, and 20 per cent. of gametangia were found to remain still ungerminated. Then the water of the drops was carefully withdrawn by a capillary pipette and fresh water was added. After 2 days germination was ascertained to have taken place in one drop, while in other drops no further germination

took place. The water was again renewed on this day. On the next day a great majority of gametangia became empty.

Thus whenever the medium water is renewed the dormant gametangia are brought to germination day after day, no matter whether the temperature is high (17°C.) or low (9°C.).

In connection with the above fact an experiment was done to ascertain whether the gametangia require a dormant period of a certain length or are able to germinate as soon as they are formed. A few resting cells, in which the formation of the sacs was already completed, were laid in a hanging drop of water. After 3 days some of the sacs produced gametangia apparently matured. On the same day the water was renewed. Next day, 2–3 gametangia were found empty and a number of gametes were found swimming about in the medium. Renewal of water was made on the same day. Next morning, the gametangia were found to have germinated by about 30 per cent.

It is therefore evident that the winter gametangium can germinate in fresh water soon after maturation. We are now able to state that the medium water, in which the resting cells are germinated, acts upon the gametangia as inhibitory of germination. Certainly, the medium has undergone a modification. Presumably a deficiency of oxygen occurred or certain substances were generated.

According to WEISS (1925) who studied the effect of soil reaction on infection in *S. endobioticum*, the range of H-ion concentration, at which infection is obtained, is PH 3.9–8.5, an optimum being nearly PH 5.0. He maintained the most favourable soil reaction to be neutral to slightly acidic. I did not go so far as to determine the limit of H-ion concentration, beyond which the germination of gametangia is inhibited. As the result of a preliminary experiment, it may be mentioned that an almost normal course of germination was observed in PH 5.8–8.4. The medium water in a glass vessel, in which the gametangia remained ungerminated, was ascertained to be alkaline, becoming stronger as the medium became older. The alkaline reaction is caused by the soluble substances contained in the glass. This reaction of the medium probably interferes with the germination of gametangia.

A further point worthy of mentioning is that the gametangium requires liquid water for germination. As an interesting fact, the resting cell germinates without liquid water. When spread over a piece of a wet silk cloth or filter paper, it could germinate as well as when immersed in water. However, the gametangia thus produced

remained ungerminated, although the cloth or paper was repeatedly washed with fresh water with a view to eliminating any detrimental substance supposed to be generated during the germination of the resting cell. Most probably, the imbibitory action of the gametangium for water is overpowered by the surface tension of water, whereby the gametangium cannot absorb it sufficiently enough to cause the germination. When the cloth or paper was so much wetted as to form a water film over the gametangium laid upon it, the germination took place as usual. This fact will justify the above conclusion.

From the foregoing experiments we conclude that the medium, in which the resting cells germinate, renders the germination of the derived gametangia impossible. It shows that the germination of the resting cell is not influenced by the chemical substances present in the medium so sensitively as that of the gametangium. The gametangium seems to require comparatively pure water, such as fresh, rain or tap water.

The resting cell is able to germinate on a sufficiently moist substratum, but the gametangium needs the presence of liquid water for germination. We infer that in the field the resting cell produces the winter sorus by virtue of moisture contained in the soil whenever the temperature and other conditions are favourable, but the derived gametangia remain ungerminated till they have rain water.

6. Vitality of the Gametangium

In connection with the preceding experiments a problem naturally arises as to how long the winter gametangia can retain their vitality.

As the material for this study the resting cells were isolated from the affected stem of the host and put in water on November 28, 1925. At the end of December a great majority have already produced gametangia, and the rest were in progress of germination. From this stock culture the gametangia were taken at times with a capillary tube, and mounting on drops of fresh water their germination was tested.

On January 25, 1926 (gametangia 40–50 days old), the germination was observed to proceed normally.

On February 16 (gametangia 62–72 days old), when tested in a room at 17°C., the germination took place in the usual interval of time. There were some gametangia which remained ungerminated for 24 hours. Kept in a room at 4°–5°C. for one or two days no germination

took place, but on the fourth day (at 7°C.) some gametangia germinated and on the fifth day (at 6°C.) numerous swimming gametes were present in the medium.

During February 19–23, the medium water was left unrenewed. On February 23, the temperature was raised to 8°–9°C. but the germination did not take place. The water was renewed on this day. On the next day innumerable gametes were produced, showing that fresh water is requisite to germination.

Lastly the germination was tested after four months. All of the gametangia seemed to have remained dormant. Most of the gametangia brought to fresh water germinated by the next day.

The experiment was then discontinued, since the material was exhausted. In high probability the vitality might be retained still longer.

It may be remarked that in the above experiment the germination was not confined to recently produced gametangia but there seems much justification for the opinion that those formed at an earlier period have remained dormant and come to germination.

The longevity of the viability seems to be influenced by temperature. Stored in warmer places the culture medium is soon spoiled with Infusoria or bacteria and the gametangia perish.

So far as observed, we see that both winter and summer gametangia retain their vitality for similar lengths of time.

7. The Gamete

The gamete derived from the winter gametangia, as compared with those from the summer ones, show nothing peculiar in their morphological and physiological characters. CURTIS (1921) pointed out in *S. endobioticum* that the gametes from the resting gametangium are larger than those from the sorus gametangium. In our fungus it is practically impossible to find such a difference.

V. The Copulation of the Gamete

1. The Process of Copulation

In the foregoing pages mention has been made of gametes encysting themselves after coming to rest. This asexual behaviour of the

gametes can be observed in every germination experiment. Typically, however, they copulate with each other and form the zygote. The copulation proceeds as follows:

When a gamete is at rest on the substratum (cover glass) or on the surface of water, a swimming one soon comes in contact with it, and for some minutes pushes against it or glides on its surface. As soon as the motion of the active gamete appears stopped, the contact surface of both gamete bodies is broken and the two bodies are fused into a spherical mass, just as the two oil drops floating on water (Fig. 4). At the time of copulation the sedentary gamete is as a rule non-

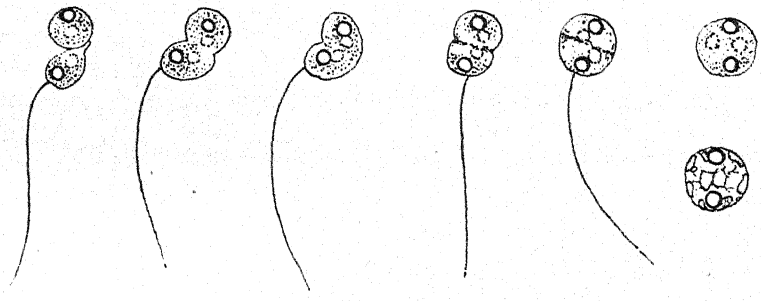


Fig. 4. Process of copulation. (×1200)

ciliate,[1] but as the active gamete is provided with a cilium, the resulting zygote is uniciliate. In most cases the zygote is not capable of swimming, though the cilium may show a gentle swinging movement. Perhaps it is due to an attachment of the sedentary gamete to the substratum. The cilium is finally dropped off preceding the encystment. The ciliated and the non-ciliated stage of the zygote is easily recognized by staining. No sign at all indicating the intrusion of the cilium into the body is found.

In a rare case a sedentary gamete is fused by an active one before its cilium is thrown off, resulting in a two-ciliate zygote. Frequently it shows a rolling movement for a while, but is unable to swim about.

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(1) Killing with the fumes of osmic acid and staining with a weak alcoholic solution of gentian violet, the presence or absence of the cilium can be exactly decided.

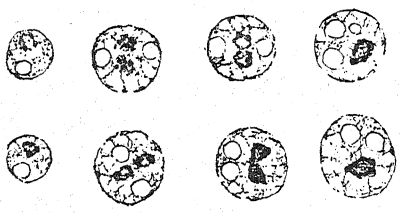


Fig. 5. Karyogamy in the zygote (fixed in FLEMMING's solution and stained with iron-alum haematoxylin).

a, uncopulated gametes just encysted; *b*, zygotes just formed; *c*, gamete nuclei approaching each other; *d*, fusion of gamete nuclei. (×1800)

The zygote proceeds to encyst itself like the uncopulated sedentary gamete. In the mean time the two nuclei approach each other, remain for a while in contact, and finally are fused into a zygote nucleus (Fig. 5). The oil-drops from both gametes lie apart, generally in an opposite position. Such an orientation of the drops is often helpful in distinguishing the zygote from the encysted gamete. The gamete with more than one drop looks, when encysted, like the encysted zygote, but the drops are unequal in sizes and are found grouped.

2. Copulation of the Gametes from the Same Summer Gametangium

The foregoing account regarding the zygote formation is based on the observations of gametes produced in the same medium from several gametangia. Therefore, it is quite uncertain whether two copulating gametes originate from the same or different gametangia or sori; in other words, whether the sexuality expresses monoecism or dioecism. In *Synchytrium endobioticum* CURTIS (1921, p. 439-440) is of the opinion that fusion takes place between gametes derived from different gametangia of the same sorus, but not between those derived from a single gametangium. In her work no statement is made as to how the behaviour of the gametes from a single gametangium has been followed out. It seems that monoecism or dioecism is not evidenced by direct observations. To make this point clear in *S. fulgens*, a single gametangium was submitted to germination in each hanging droplet, and observation was made upon the sister gametes. Their behaviour was quite the same as observed in the foregoing experiments and the copulating action was manifested normally. The fusing process has been observed several times without any difficulty. *S. fulgens* is therefore monoecious (paedogamous according to HARTMANN, 1927,

p. 451). As to whether the copulation between gametes from different gametangia or sori takes place more easily, we cannot decisively state. From the fact that 30–40 per cent. of sister gametes may enter into the formation of the zygote the monoecious sexuality may be considered as being ordinary.

3. Copulation of the Gametes from the Resting Cell

In the process of copulation, as in other behaviours, the gametes derived from the winter gametangia precisely accord with those from the summer gametangia. When the medium is exposed to a temperature of 20°C., we can find a fairly large number of zygotes. The process of fusion is directly observed under the microscope.

The zygote formation is also found among sister gametes, so that monoecism is also ascertained in the winter cycle.

In *S. endobioticum*, CURTIS (1921) does not mention the zygote formation by the gametes from the resting cell (resting sporangium), and it is often believed (KÖHLER, 1927, p. 148) that the gametes derived from the resting cell exclusively develop into the summer sori. As will be mentioned later (next section), the zygote of *S. fulgens* develops into the resting cell as in *S. endobioticum*, so it follows that the generation of the resting cell may be directly succeeded by a similar generation.

4. Behaviour of the Gametes towards Copulation

As the gametes are indeterminate in copulation, it seems necessary first of all to follow out closely the behaviour of individual gametes before we attempt to get a deep insight into the nature of sexuality. The following may be mentioned as the important points upon this matter.

1. Of first importance is the recognition of the sexual reaction in the behaviour of the gametes. Preceding the fusion active gametes are attracted by a sedentary one and usually present for some minutes a pushing movement against the latter, or a gliding movement on it. However, the given behaviour fails in some gametes; meeting the same

sedentary gamete they appear quite indifferent of fusion, not remaining in contact with it but being in forward movement. Here the sexual reaction cannot be recognized.

2. The sexual reaction may be manifested in different degrees. Some gametes meeting with a sedentary one are not quite indifferent but for a short time stay there, exhibiting meanwhile the reaction as noted above, then rapidly fly away. Other gametes stay at the same sedentary one much longer—10 minutes or more—, attending, as it appears, to fusion. Such a difference in the duration of stay may perhaps be due to the different degrees of the sex character acquired by the active gametes.

3. While it is usual that the active gamete reacting to the sedentary one ultimately departs, if not successful in fusion, it frequently happens that the similar gamete, after reacting in an usual manner to the sedentary one, becomes immovable and there encysts singly.

4. The first visitor to a sedentary gamete stays for some minutes but being unsuccessful in fusion flies away. The second and third visitors do the same. Lastly a fourth visitor may effect fusion. In other cases several visitors are found staying together at a sedentary gamete, all engaging in fusion, and a later-coming visitor may effect fusion.

5. A visitor to a sedentary gamete, after an attempt at fusion for a long duration, comes finally to rest close to it. After a while another active gamete visits a pair of the sedentary gametes and expresses the sexual reaction equally to both of them. In a shorter or longer duration it fuses, not with the original gamete, but with the first visitor.

Sometimes several active gametes are found engaging in fusion with a sedentary gamete. Gliding movements may be seen among themselves and on the sedentary one. No one is, however, able to fuse with the original sedentary gamete. On the other hand, copulation may be effected between two visitors.

6. When one of several active gametes together engaging in fusion with a sedentary one effects fusion, all the rest soon fly away at once or in rapid succession. To the zygote thus formed no visitor is afterwards found. Rarely, it happens that active gametes come to a

just-formed zygote, appearing as if engaging in fusion, but shortly after they fly away.

7. Rarely it is observed that two gametes attain simultaneously to the resting condition close to each other, and, while one of them is soon visited by one or more gametes and transformed into a zygote, the other remains solitary or receives the visitor less frequently and finally goes to encyst singly.

8. The sedentary gamete at first receives active visitors very often, but later active gametes passing near by pay no visit to it.

The significance of the behaviour of gametes so far given will be considered in later sections.

5. Conditions of Copulation

In the nature of reproduction the gamete under consideration is said to be dual; it performs sexuality, otherwise it is able to develop further asexually. Considered physiologically, we can say that every gamete represents a combination of the gamete and the zoospore. It gives us an idea that the manner of reproduction is here very labile and it appears to be easily controlled by internal or external conditions. With this view some experiments were carried out.

A. External

a. Temperature

As already mentioned, temperature exerts an influence upon the germination of the gametangium and upon the behaviour of the liberated gametes. Expecting the existence of a certain relation between temperature and the formation of the zygote, the gametes were liberated at different temperatures and their behaviour for copulation was carefully observed. For this purpose gametangia from the same diseased patch were submitted at the same time to germination at different temperatures. The results of repeated experiments may be recapitulated as follows:

A highest percentage of the zygote is obtained when the gametes are at a temperature of 20°C. or thereabouts. It may be noted that the same temperature is, as already stated, most favourable for the germination of gametangia.

At higher temperatures, viz. 24°–26°C., the liberated gametes are less active and their behaviour is not in favour of copulation. Yet, within the range of the said temperatures the zygote formation is not absolutely excluded, though it may be reduced considerably with increasing temperatures.

At lower temperatures the activity of the gamete, as already mentioned, is not lessened, but the percentage of the zygote is reduced. The reduction is slight at 16°C. but considerable at 10°–12°C. Exclusion of the zygote is impossible even at 8°C., a temperature near the minimum for the germination.

The accompanying table (Table V) will show a reduction of the zygote formation at lower temperatures. In each culture experiment the gametangia taken from the same diseased patch were submitted to germination, each in a drop of water at a temperature of 7°–8°C. and as a control at the optimum temperature (19°–20°C.). In each experiment 10–20 drops were examined and the mean number of the zygotes produced by the gametes from one gametangium was determined.

TABLE V

Showing a mean number of zygotes produced by the gametes of a single gametangium at optimal and lower temperatures.

No. of experiment	Number of zygote (19°–20°C.)	(7°–8°C.)
1	44	23
2	38	18
3	35	15.3
4	22	14
5	34	17.6
6	33	12
7	28	16

The influence of temperature upon the formation of the zygote may be observed more distinctly by changing the temperature of the medium. The gametangia were first germinated at 18°–19°C. Standing for 3 hours at that temperature the liberated gametes were going to form prosperously the zygote in succession. Exposing soon the medium to 26°C. the copulation among the existing gametes became

remarkably less frequent as compared with those remaining at the original temperature. In a reversed manner the gametangia were germinated at 26°C. In 3 hours a large number of gametes were liberated, but the process of copulation was not easily recognized. Exposing then the medium to 20°C. the gametes were restored to activity in forming the zygote.

It may be remarked that as regards the copulation at higher temperatures the gametes behave differently according to whether they are formed at an optimal or supraoptimal temperature. Those formed at a higher temperature and swimming in the medium at the same temperature are not likely to copulate. On the other hand, when the gametes are formed at an optimal temperature (20°C.) and their medium is exposed to a higher temperature, the copulation occurs more frequently.

From this fact we induce that the effect of supraoptimal temperatures upon the formation of the zygote is twofold ; on one hand it gives rise to gametes unable to copulate and on the other it interferes with the sexual act of the normally developed gametes.

Now, to make the condition of copulation more comprehensive, the relation of temperature to the copulation, to other behaviours of the gametes, and further to the germination of gametangia is recapitulated below. Optimal (20°C.), supraoptimal, and infraoptimal temperatures to germination are denoted by *O*, *H*, and *L* respectively, and the corresponding temperatures in the medium of the gamete by *o*, *h*, and *l*.

1 ($O \times o$): Germination earliest; resting of the gamete earliest; zygote most numerous.

2 ($O \times h$): Germination as in 1; below 27°C. the gamete active and its period of rest retarded, copulation frequent; but above 27°C. it settles down and disorganizes without encystment; zygote almost none.

3 ($O \times l$): Germination as in 1; swarm period prolonged; zygote reduced in number.

4 ($H \times o$): Germination checked or retarded; below 26°C. the gamete normal and active, and swarm period and zygote formation nearly as in 1; above 26°C. the gamete sluggish and often compound, settling soon down, and zygote few or none.

5 ($H \times h$): Germination as in 4; the gamete abnormal and sluggish, settling soon down; zygote rare or none.

6 ($L \times o$): Germination retarded; swarm period and zygote formation as in 1.

7 ($L \times l$): Germination as in 6; swarm period prolonged; zygote reduced in number.

From the above it will be seen that the temperature both during the formation of the gametes and during their activity influences copulation. The gametes formed at, and exposed to, the optimal temperature (20°C.) are likely to copulate, but those formed at subraoptimal temperatures or exposed to extraoptimal temperatures are less capable of copulation.

b. Medium

In the course of observations on the copulation of gametes in the hanging drop of water, it has come to my notice that at an early period of the observation the zygotes exceeded the azygotes in number, but, as the time advanced, more azygotes were formed than the zygotes, making a reversal of the relative number of both forms. This was clearly manifested by rotating the slide carrying the hanging drop at intervals of 30 minutes. During the first 30 minutes after the beginning of germination most gametes assembled at the brightest area of the drop and they were going to form there both zygotes and azygotes. When the slide was rotated to a certain angle, the swimming gametes came to a new brightest area and within 30 minutes some of them settled there as zygotes and azygotes. By this means the decreasing percentage of the zygote during the first, second and third intervals of 30 minutes was clearly presented. This fact suggests that the medium undergoes, as the time elapses, a certain change interfering with the copulating action of the gamete. The following experiments were conducted to know about the matter concerned.

1. As the medium of germination two sets of the hanging drops of water were prepared. In one set fresh tap water was used, while in the other the water-drops, in which gametangia were germinated the preceding day, was again used. In both sets the germination occurred at the same rate, and in succeeding 2 hours the number of resting individuals (zygotes and azygotes) were approximately the same. However, in the zygote formation the set of the fresh water has apparently surpassed the other set. At later examinations this difference became obscure.

2. The gametes were liberated in drops of fresh tap water. When they were produced in great abundance, distilled water was added to some of the drops. The formation of the zygote took place

more pronouncedly than in the remaining drops. In some drops cane sugar was introduced, making the medium to 1.5 or 2 per cent. of it. Active gametes soon began to settle down. Gametangia about to germinate were restrained from liberating the gametes. The formation of the zygote was greatly prevented.

3. In hanging drops of tap water, in which a mass of gametangia was submitted to germination 2 or 3 days before, there were found still a certain number of ungerminated gametangia. They were transferred to a new drop of water. Within 2–3 hours they germinated just as in the case when using fresh gametangia, and the behaviour of the gametes appeared quite normal, being capable of copulating with easiness. Meanwhile the gametangia remaining in the original drops did not germinate.

4. In a hanging drop, on which gametangia were sown on the preceding day, numerous swimming gametes were found. They seemed to have been derived recently. With the aid of a capillary pipette they were transferred to a drop of distilled water. As a remarkable fact, the gametes in the new medium came sooner or later to rest and proceeded to form the zygote in a large number. During the same interval the gametes remaining in the original medium could not behave as ordinarily, and a greater part of them settled down, so that the number of the zygote formed there was far less than in the new medium.

These experiments suggest that the difficulty of forming the zygote by the gametes which are derived at later periods may be connected with the generation of certain substances or with deficiency of oxygen.[1] As to the generation of the substances attention is called to a swelling action of the germinating gametangia. This action is caused by the generation of water absorbing substances in the gametangium. On germination each gametangium gives them off into the medium, so that their amount will be increased with increasing number of germinated gametangia. The presence of such substances was proved by testing the medium with FEHLING's solution, by its being easily reduced. These substances would likely have some influence upon the behaviour of the gametes. Furthermore, it is conceivable that certain metabolic products generated by the gametes may accumulate in the medium. It is, however, not certain how much they do interfere with the copulating action of the gametes.

(1) Under a cover glass or in a capillary tube the behaviour of gametes is otherwise than usual, showing evidently deficiency of oxygen in the medium.

In support of this view further experiments were carried out.

5. On the cover glass, after being finely sprayed with water, gametangia were dusted. By this means we could obtain fine drops of water of varying sizes, each containing a single gametangium. In smaller drops the germination was retarded considerably or sometimes impossible. Often the gametes could not get out of the ruptured gametangium, as they were not able to swim about. It is considered that the surface tension of water surpasses the power of absorbing water by the gametangium and also certain soluble substances generated in the gametangium are diffused on bursting into the medium and prevents intake of water by the freed gametes. Except for these extreme cases the liberated gametes presented a jerking movement within a small area of each drop, giving them good occasion to meet one another. However, in smaller drops it was often the case that the gametes were dull in movement, settled down to the bottom of the drop, and were unable to encyst normally. Under such circumstances the copulation occurred less frequently in smaller drops, as may be seen in the table given below (Table VI):

TABLE VI

Showing the number of the zygote by the gametes from a single gametangium in large and small drops at 21°–22°C.*

Small drop			Large drop		
No. of drop	Number of zygote	Remark	No. of drop	Number of zygote	Remark
1	0	Only 3 gametes liberated.	1	few	
2	0	Only a few gametes liberated.	2	6	Gametes mostly settled down.
3	1	Gametes soon settled down.	3	7	
4	3		4	12	
5	5		5	13	
6	5		6	17	
7	5		7	17	
8	5		8	17	

* The drops having a diameter less than 5 times that of the gametangium are denoted by "small" and all others by "large."

TABLE VI—*Continued*

Small drop			Large drop		
No. of drop	Number of zygote	Remark	No. of of zygote	Number of zygote	Remark
9	few		9	18	
10	few		10	19	
11	few		11	20	
12	few		12	20	
13	few		13	20	
14	6		14	22	
15	7		15	22	
16	8		16	26	
17	10		17	28	
18	10		18	30	
19	10		19	37	
20	12				
21	12				
22	13				
23	13				
24	14				
25	15				
26	15				

Thus in large drops more than 15 zygotes are usually formed, while in small drops they do not exceed 15 in number, being mostly less than 10. The reduction of the zygote formation in small drops may be explained by assuming that the soluble substances given off from the gametangium are more concentrated in small than in large drops.

6. In order to eliminate the substances which spoil the medium, the following method was devised. Using a hollow slide, gametangia were placed on the hollow and over it was stretched a sheet of filter paper, being tightly pressed with a glass ring. The slide was then immersed in running water at about 20°C. The gametangia could germinate in the hollow space and the derived gametes remain there, while the medium water was constantly renewed. Examining after a few hours or on the next day, the zygote was found to surpass the azygote in number.

The results of experiments so far carried out show that the medium water of the gametes becomes unfavourable for the formation of the zygote when the number of the gamete are increased.

The conditions under which the gametangia germinate in the field are not of course always similar to those of the hanging drops of water used in the experiments. However, it may be true that the rosette leaves of the host, on which the gametangia are to germinate and the infection is to be effected, is spoiled with dust, soil particles, or substances precipitated from the exudation water. Unless sufficient rain water makes the surface of the host clean, the liquid water upon the host is not a medium favourable for the formation of the zygote.

B. Internal

a. Age of the Gametangium

The young sorus is mucilaginous and on pressing it the mass of gametangia is released in a viscous consistence. Withdrawal of water takes place in the following one or two days, making the sorus pulverous. At this stage the gametangia are said to have attained maturity. This can be recognized with the aid of a hand lens by its becoming a little lighter in colour.

Then follows the dehiscence of the sorus. Whether it takes place sooner or later depends upon the condition of the host plant, chiefly upon its water content. We may bring it to an earlier dehiscence, if the host plant is kept sufficiently moist under a bell jar and its turgidity is increased. In the field a rainy day or a cool night accelerates the dehiscence which may also be caused when the host withers; as owing to the shrinkage of the tissue surrounding the host-cell, the outer exposed wall of the latter is broken and the gametangia are exposed from the sorus.

According to weather conditions the mature sorus may remain undehisced for 2–3 days. In consequence the dehiscence of the sorus is not an exact index showing the period of maturation.

(1) *Gametangia on Different Days after Maturation*

To know whether the age of gametangia shows a certain relation to the copulating action of the gametes derived from, the gametangia were submitted to germination on different days after they have

matured. The material taken from just matured but not yet dehisced sori and from those on the day of dehiscence and on one day to a few months after dehiscence was examined.

In general, the percentage of the germinative gametangia was decreased when they became older.(1) Yet, whenever the gametes were abundantly liberated in a medium at a temperature of 17°–20°C., they were able to form the zygote. The percentage of the zygote presented variations according to materials examined, but it was by no means correlated with the age of gametangia.

(2) *Different Gametangia from the Same Sorus*

CURTIS reports in *S. endobioticum* that the gametangia jerked out from a dehisced sorus precede as regards the maturation period those remaining at the bottom of the sorus. According to her, the germination takes place soon in the freed gametangia while it is retarded for 36 hours in those remaining in the sorus. If such be the case in *S. fulgens*, different gametangia of the same sorus, when brought to germination at the same time, would afford certain facts regarding the zygote formation in connection with the age of the gametangia.

A small piece of an affected leaf, upon which a few sori already matured but not yet dehisced were found, was placed in water. After 20 minutes some sori dehisced and some gametangia were dispersed in water. Soon the freed gametangia were transferred to a hanging drop of water. Further, epidermis was removed from a diseased leaf and cut into small pieces, each carrying 2–3, dehisced or undehisced sori. Each piece was then brought into a hanging drop. In this manner I proceeded to compare the feature of copulation in gametes derived from the gametangia freed of the sorus and from those which remained in it.

The hanging drops which contained the freed gametangia yielded a larger percentage of germination in $1^1/_2$ hour. During the same interval of time, in another drop containing a piece of epidermis appeared numerous gametes from some gametangia which remained in the dehisced sorus. During an interval of 4 hours, however, a great majority of gametangia in the sorus remained still ungerminated.

Thus we have found that the gametangia jerked out from the dehisced sorus begin to germinate earlier than those remaining inside

(1) See the experiments upon the viability of gametangia recorded in the preceding pages.

the sorus. This may support the view of CURTIS on the difference of the maturation period in different gametangia of a sorus. Therefore, attention was paid to the zygote formation with these younger and older gametangia.

The sori remaining undehisced in water for a few hours may be assumed to be younger than already dehisced ones, and when they come to dehisce in water, the freed gametangia may be said to have been subjected to germination as soon as they mature.

Hanging drops containing the said materials were examined on successive days, renewing the water each day. During the first three days the gametangia remaining at the bottom of the dehisced sori have germinated in succession, but on further days no germination was observed. Among the gametes liberated on the 2nd or 3rd day numerous zygotes have been formed.

The sori which remained undehisced during the day of immersion into water have set free the gametangia, some on the 2nd day and some on the 3rd day (exceptionally on the 4th day). A great majority of these gametangia started to germinate on the day of dehiscence, though a few yet remaining inside the dehisced sori did not germinate till the next day or a later date. The copulation of these gametes was effected as easily as seen in the gametes derived from the gametangia which were freed of the sorus soon after immersion in water. Under favourable conditions 50 per cent. of the gametes went to form the zygote.

In the experiment we see that the gametangia which are no sooner subjected to germination than they mature, produce the gametes whose copulating activity is quite normal.

Thus the conclusion to be arrived at regarding the relation of age of gametangium to the copulation of the derived gametes may be as follows. The fresh gametangia taken from just dehisced sori give a good result in obtaining a highest percentage of the earlier germination and of the zygote formation as well. The gametangia exposed on the surface of the host plant tend to germinate at variable rates, generally being delayed. In the same medium association of gametangia having divergent rates of germination results in a less frequency of copulation.

In *S. endobioticum* (CURTIS, 1921) it has been reported that the gametes capable of copulation are obtained from the gametangia which remain for some days after maturation, while those liberated from just matured gametangia do not copulate. The same thing is known in an allied fungus, *Olpidium* (KUSANO, 1912). In a recent study on this

genus (KUSANO, 1929 a) a premature stage is marked regarding sexuality in the germinative gametangium; the gametes derived from the gametangium which have just attained a germinative stage soon tend to settle down in the medium and are not able to copulate. In *S. fulgens* it has been shown that the gametangium, as soon as it attains a germinative stage, is able to liberate the gametes capable of copulation. Therefore, the premature stage as observed in *Olpidium* and *S. endobioticum* is not ascertained in our fungus.

b. MUTUAL RELATION BETWEEN THE GAMETES

(1) *Duration of the Copulating Activity*

As already noted, one of the copulating gametes is at rest, while the other is actively swimming. Coming to rest is not a characteristic confined only to certain gametes, as every gamete sooner or later attains the resting period previous to encystment. It is evident that this condition of the gamete indicates the sexual action; in other words, the gametes cannot manifest the sexual action, unless a critical period arrives upon them.

After coming to rest, all individuals proceed under ordinary circumstances to encyst. Presumably, it appears that they would become incapable of copulation, if the encysting process advances further.

Taking these points into consideration the time relation of the gametes to fusion was observed. For this purpose a mass of summer gametangia was sown on a hanging drop of water at about the optimal temperature (20°C.). Among innumerable swimming gametes in view in a fixed field under the microscope the appearance of sedentary ones was awaited. After noting the time of their rest, the period and the process of copulation were carefully observed on each.

1. At 20°C. germination began after three hours. 25 minutes later, the formation of the zygote was first observed. During 27 and 30 minutes after beginning of the gamete liberation an increase of sedentary gametes became conspicuous. Among them seven individuals, in which the time of their rest was known, were selected and their conduct was noticed.

Gamete *a*:—During an interval of 6 and 13 minutes after rest it was visited several times by active gametes which all darted away after attempting fusion. Immediately another visitor came upon it and after engaging in fusion for 4 minutes a zygote was formed by the two.

Gamete *b* :—During an interval of 6 and 13 minutes after rest it received active gametes several times but remained unfused. During the subsequent one hour frequent visits of active gametes were observed, but no fusion took place at all.

Gamete *c* :—The fusion was effected 19 minutes after rest.

Gamete *d* :—An active gamete fused with it 3 minutes after rest.

Gamete *e* :—It formed the zygote 6 minutes after rest.

Gamete *f* :—Several visitors tried to fuse with it during an interval of one hour after rest but all in vain.

Gamete *g* :—During an observation for one hour none of several visitors could fuse with it.

Thus some sedentary ones (*b*, *f*, *g*) were incapable of fusion with any active visitor. Notwithstanding, they displayed the gametal nature, as might be seen from their attracting swimming gametes.

2. Germination took place at 19°C. after 1 hour and 27 minutes. In the succeeding 2 minutes a vast number of gametes were liberated, showing the germination of several gametangia at once.

Gamete *a* :—It came to rest at 10 minutes after germination began, and soon received two visitors at the same time. 6 minutes later, it fused with one of them.

Gamete *b* :—It came to rest at 18 minutes and was immediately visited by an active individual which effected fusion 6 minutes later.

Gamete *c* :—It came to rest close to and nearly at the same time with *b*. Soon 3 active gametes visited it, but all darted away after 7 minutes.

3. Germination took place at 19°C. after 1 hour and 35 minutes. 45 minutes after liberation of gametes, a zygote has been found. Immediately observation was made on the following 5 individuals of gametes coming to rest:

Gamete *a* :—The zygote is formed at 6 minutes after rest.
Gamete *b* :— ,, ,, 6 ,, ,,
Gamete *c* :— ,, ,, 8 ,, ,,
Gamete *d* :— ,, ,, 6 ,, ,,

Gamete *e* :—Immediately after rest it was visited by an active gamete which was endeavouring for fusion during some minutes. Soon it was again visited by a second gamete and after 6 minutes it effected fusion with the latter visitor.

In the case of the last individual (*e*) we find that no difference is revealed in the behaviour towards copulation between the first and

second visitors. It may be assumed that the condition of the plasmic membrane of both gametes, *e* and the first visitor, is not fitted for fusion.

4. The gametangia were brought to germination at 22°C. The germination began after 1 hour and 30 minutes, and the zygote formation took place more numerously than in the case of others. In this experiment the sedentary gamete was first found at 50 minutes after germination, being later than usual. The following 5 gametes came successively to rest and were observed in the act of copulation.

Gamete *a*:—It became the zygote at 5 minutes after rest.
Gamete *b*:— ,, ,, ,, 4 ,, ,, ,,
Gamete *c*:— ,, ,, ,, 5 ,, ,, ,,
Gamete *d*:— ,, ,, ,, 10 ,, ,, ,,

Gamete *e*:—It was visited by an active gamete first at 29 minutes after rest. After trying for fusion for 2–3 minutes the visitor came to rest. At this instant a second visitor was found in association, making a gliding movement on both gametes at rest. After 4 minutes the zygote was formed by the first and second visitors, while the original gamete remained unfused with any subsequent visitor during a further interval of one hour.

It is a remarkable fact that the gamete *e* received the first visitor laterly than *d*, though both came to rest at the same time and close to each other. Numerous active gametes were passing close to them, and while *d* was receiving visitors all attempting fusion, *e* remained solitary; occasionally active gametes came to *e*, stopped suddenly, but immediately dashed away, showing no attempt at fusion. Finally, at 29 minutes after rest, as mentioned above, a visitor appeared trying to fuse but in vain.

Why the difference in the fusing activity is revealed between *d* and *e* is difficult to explain. Perhaps the attractive action of *e* for active gametes was too feeble or was exerted too late, and at that time the encysting process had advanced so far as to be incapable of breaking the plasmic membrane for fusion.

From the foregoing and further unrecorded experiments we may draw the following conclusion:

Sedentary gametes immediately attract active ones and effect copulation in the interval between a few and about 20 minutes after coming to rest. Beyond this period their attractive action becomes feeble and their fusing capacity is lost.

Between the sedentary and active gametes there may occur proper and improper combinations for insuring copulation.

Rarely there occur sedentary gametes which have no ability to attract the active ones.

(2) *Aggregation of Gametes and Period of their Rest in Relation to Copulation*

The most characteristic feature which points to a certain mutual relation between the gametes regarding the presentation of sexuality is their aggregation at certain spots in the medium. Generally this feature is not observable, when the gametes are densely liberated from numerous gametangia. Submitting, however, a single or two gametangia to germination in a small drop of water it can be pronouncedly exhibited. At first it was thought that the aggregation might have been caused by the phototactic reaction, the gametes assembling and depositing at the brightest area of the drop. This was, however, disproved by an indefinite orientation of the aggregate in several drops lying on the same cover glass. After a careful examination it has been ascertained that the sexual attraction is concerned in this respect.

The gametes are all very active during a certain interval of time after liberation. When one of them is inclined to become sedentary, the active gametes, generally one or two, but sometimes more, come in contact with it, show a fusing action for some minutes, and if one of them effects fusion, the other departs, leaving behind the zygote alone. In this way, minute after minute, the zygote is increased in number in a scattered state. Sooner or later, according to individual variations of gametangia, a majority of gametes are attaining the sedentary condition almost simultaneously or in rapid succession. At this critical period the mutual relation between them brings forth a remarkable effect. A pioneer at rest attracts the active gametes which stay there engaging in fusion. In the mean time some of them become sedentary and attract those still remaining active. Thus within a few or 10 minutes many gametes formerly swimming about in the medium freely flock to a spot occupied by a first sedentary gamete, some still swimming and some depositing. In the crowd copulation is taking place between the motile and immotile gametes, and there the zygote is increased in number. Often some motile partners become immotile previous to effecting copulation and they become an obstacle to the active gametes

intending to approach the other immotile partners. In consequence, some of the sedentary gametes in the crowd are obliged to encyst singly.

We have previously pointed out that an approximation of active gametes to a sedentary one is enabled by an attractive action of the latter. During the formation of the aggregate this action is exhibited more emphatically. The active gametes formerly found uniformly distributed in the medium are gathering at the area where sedentary ones are deposited in group. Tracing the route of these active individuals it is observed that, when they reach the aggregate, they stop in contact with sedentary gametes or wander about through them. Whenever they swim away from the aggregated area and reach a point at a certain distance from it, they change the forward direction and at length they come back to join the aggregate again. Thus the active gametes which once entered the aggregated area are obliged to stay there and in the mean time they enter into the formation of the zygote with the sedentary ones, or else they become sedentary. Without any doubt the mode of their reaction expresses phobotaxis. Most probably it is caused by a specific chemical stimulant secreted by the sedentary gametes, representing phobochemotaxis. Otherwise, the stimulant might be of a physical nature, of which, however, we know but little. In either case it is true that the attraction zone is small and the attraction is feeble when only a single or a few sedentary gametes exist, but a wider zone with strong attraction may be formed round the aggregate of numerous gametes.

The exhibition of attraction by the sedentary gamete appears to be limited in duration. This is manifested by the behaviour of swimming ones which retain their activity longer. After they swim about for a certain interval of time within the attraction zone, often stopping in contact with the sedentary gametes, they leave the zone and wander again freely in a wider area of the medium. At length the aggregate remains without any active member.

Assuming chemotaxis as the cause of aggregation, this phenomenon may be induced in connection with the following conditions. Firstly, the sedentary gametes cease to secrete the stimulant on becoming older. Secondly, the stimulant in the attraction zone is diffusing and diluted. Thirdly, the sensibility of the swimming gametes to the stimulant is becoming weaker.

On the whole, what we learned from the aggregation of gametes supports the statement previously given regarding the sexual act of

the gamete, viz. the sedentary gamete attracts the swimming one for copulation; the swimming one does the same on becoming itself sedentary; and exertion of the attraction is limited in duration.

Attention has been directed towards the influence of external factors upon the formation of aggregate. As already given, infra-optimal temperatures act upon the gametes as hindering copulation. Yet on forming the group, the gamete behave quite similarly at an optimal and at far lower temperatures (7°–8°C.). In other words, reciprocal relation between the sedentary and swimming gametes for approximation are not altered, and an attempt at fusion by the swimming gametes is expressed normally, though actual copulation is more or less disturbed. It shows that lower temperatures give no interference with an attempt of the gametes at copulation but render their actual fusion difficult.

Any condition, under which the gametes are sluggish in movement, soon settle down, or are not able to encyst, prevents the aggregation of gametes.

Now we proceed to consider the relative length of the swarm period as an internal condition of copulaton. For this aim the study of the culture of a single gametangium gives an advantage, as the length of the swarm period can be measured in each of the sister gametes.

It has already been noticed that the gametes becoming sedentary shortly after liberation, while most of the gametes are still in great activity, are generally successful in copulation. Later, there arrives a period, at which swimming gametes become by a large percentage sedentary in aggregation. Some of them may form the zygote, but many of them go to encyst singly. The failure of copulation in this case is assigned to the fact that the individuals reacting to the sedentary ones soon become themselves sedentary and on account of a dense aggregation some sedentary ones fail to have swimming partners with which to form the zygote.

The period at which the aggregate of the sedentary gametes is formed varies with individual gametangia. It attains in some at about half an hour (Fig. 6, *II*) and in others at 1–1½ hours after germination (Fig. 6, *III*). At any rate these gametes show a slight difference from one another in the length of the swarm period. To form the zygote they must be visited by gametes having far longer swarm periods. But when such gametes are few, most of the sedentary gametes go to encyst singly (see Fig. 6, *II* and *III*).

Relating to the fact that lately sedentary gametes yield a less percentage of the zygote, the present observations give ground for the

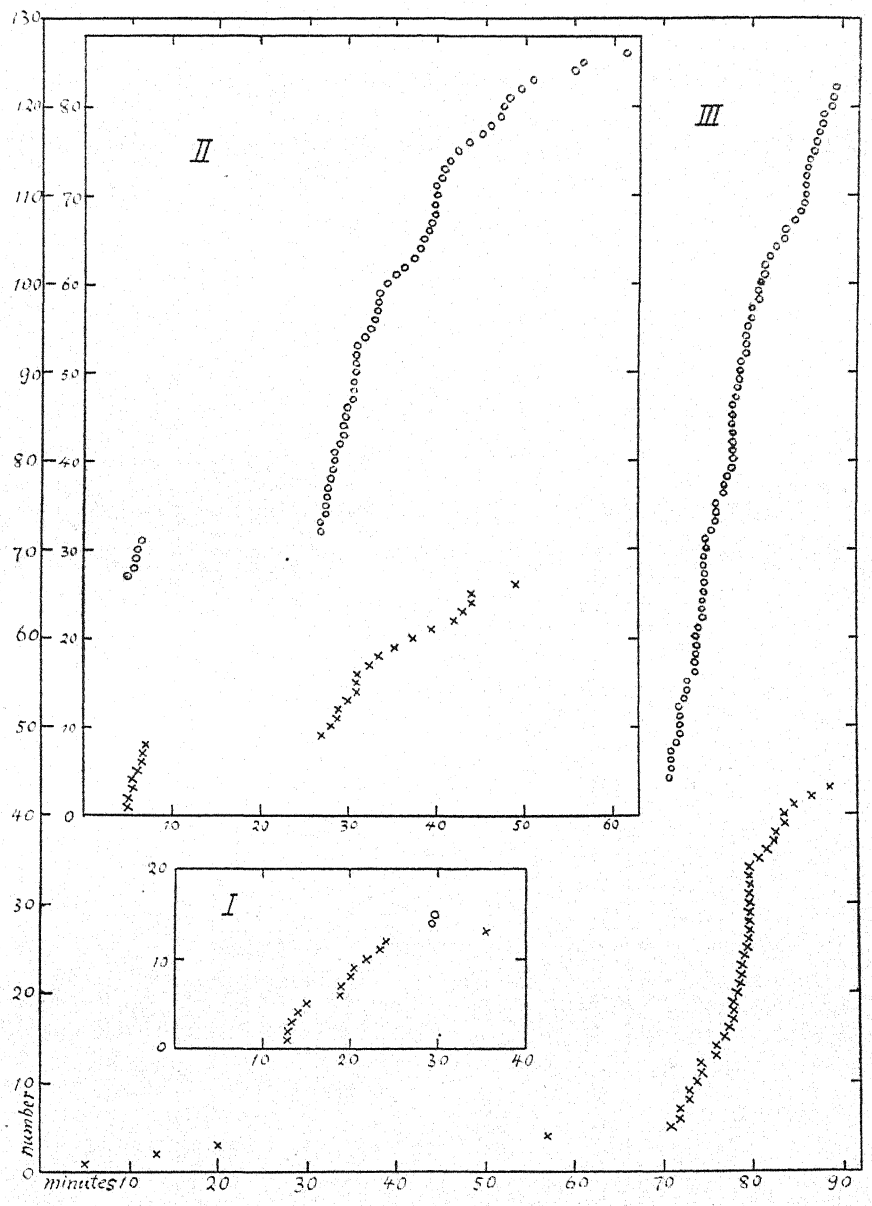


Fig. 6. The period of forming the zygotes (×) and azygotes (o) observed in each culture of a single gametangium during an interval of 90 minutes. *I*, zygote 13 and azygote 2 among ca. 250 gametes; *II*, zygote 26 and azygote ca. 60 among ca. 120 gametes; *III*, zygote 43 and azygote ca. 79 among ca. 180 gametes.

view that the modified condition of the medium is not the sole cause. In connection with the relative length of the swarm period the lately sedentary gametes are less sure of getting appropriate mates.

The behaviour of the swimming gametes is observed to be somewhat different at an early period compared to the later period, when they are disposed to form the aggregate. At the former period they react to the sedentary ones in great activity, giving us an impression as if they are very vigorous or trying fusion with an effort. However, at the later period their sexual reaction is less active, appearing as if they become exhausted of their vigour. I take the less activity as being expressed by the gametes, upon which the sedentary period is approaching, unless the medium on becoming older affects their behaviour.

Upon consideration of the swarm period it can safely be stated that the relative length of the swarm period in each two gametes has an important bearing upon their copulation, the less the difference in length, the less is the assurance of copulation. The smaller percentage of the zygote formed by the aggregated gametes will afford evidence for this statement.

In several drops lying on the same cover glass, that is, being under the same condition, the percentage of the zygote formed in each drop by the sister gametes varies considerably. The chief cause of this variation can be explicable when we take into consideration a great variation of the relative length of the swarm period in the sister gametes. Within the limit of that period, which varies, as already mentioned, according to individual gametangia, a certain number of gametes become sedentary periodically, or only a few close the period very early, while a large number become sedentary after $^1/_2$—1 hour within an interval of 20–30 minutes. In short, the relative length of the swarm period renders the copulation easier in some gametangia and difficult in others (see Fig. 6 and experiments given below).

On a mass culture the gametes, being derived at different periods from different gametangia, may usually be at different ages. Hence young and old individuals of the sedentary and active gamete are associated together in the same medium. This condition gives a good chance to each gamete to get an appropriate partner to fuse with. It must, however, be borne in mind that the medium may be spoiled with the stimulant secreted by the sedentary gamete, the amount of which is increased with the increasing number of such gametes. Therefore, at a later period the existing swimming gametes would become less sensitive towards the attractive action of the coexisting sedentary

gametes. This condition may interfere with the formation of the zygote.

Some of the experiments, which afford the basis for the foregoing statements regarding the mutual relation between the gametes, are given in the following. In each experiment the time of germination is first noted and the behaviour of gametes is observed at intervals.

1. Temperature 17°C.

7:* A few are at rest, being reacted by active ones and forming 2-3 zygotes.
12: Active ones are reacting to sedentary ones.
30: Sedentary ones not increased in number.
42: Resting individuals 12, of which more than one half are the zygotes.
62: Resting ones not increased.
72: At one side of the drop a few groups of gametes appear, each consisting of a sedentary and 2-5 active ones, and there the zygote is being formed.
77: New groups appear.
82: Active gametes are departed from most of the groups, leaving there only the zygote.
92: New groups appear and there 4 zygotes are formed.
102: Active gametes leave these groups; new groups appear at other places, forming there 2 zygotes. The observation is discontinued.

Thus, during an interval of about one hour and a half the zygote is formed periodically, amounting in total to about 23.

2. Temperature 17°–18°C.

On germination the gametangium releases a mass of immotile gametes.

5: They swim about actively; jerking gametes yet remain in the gametangium.
15: Some come to rest, by which active ones are attracted to form the group.
20: 2-3 zygotes are found in the group.
25: The zygote amounts to 12 in number; the group disappears.
35: 18 zygotes and 2 sedentary gametes are found.
45: No addition of the zygote and the sedentary gamete.
75: do.
125: do. Observation is discontinued.

On the next morning (more than 16 hours after germination) only 5 gametes are swimming. The zygote amounts in total to 35. It shows that 17 zygotes have been produced during the night, at least later than 2 hours after germination, while 18 zygotes were already formed during an interval from 20 to 35 minutes after germination.

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*In this and following experiments numerals show the time after germination (in minutes).

3. Temperature 17°C.

The gametes are liberated nearly at the same time in several drops on the same cover glass.

In most of the drops the gametes continue, except a few in each drop, the swimming movement for 24 hours. In one of them the gametes behave as follows:

25: All gametes swimming.
55: do
185: do
225: A few gametes form a small group.
245: Only 3 sedentary gametes are found in the group. To one of them comes an active gamete, but is departed after 6 minutes.
275: One of 3 sedentary gametes is found transformed into a zygote.
305: No other gamete comes to rest. Observation is discontinued.

In this case it is shown that the swarm period lasts longer than usual. A pioneer zygote has been formed on 4 1/2 hours after germination.

4. Temperature 17°C.

Two drops on the same cover glass were observed.

(1)

10: Only a few gametes remain swimming, the others having all become sedentary.
22: A few are yet slowly swimming.
40: 2 individuals are swimming.
50: Only one is found to be swimming. 37 zygotes are counted.

(2)

8: Numerous gametes become sedentary, most of them accompanying active ones.
14: Definite groups are formed by sedentary and active gametes.
23: 18 zygotes are found.
28: Active gametes are considerably reduced in number.
58: No active gamete exists; 30 zygotes are found.

The two gametangia examined belong to the short-swimming type and the derived gametes mostly become sedentary in 10–30 minutes after liberation.

5. Temperature 17°C.

On a cover glass there are found some drops, in which the gametes swim for a longer duration, while in most drops they come to rest within 1–2 hours. The zygote formation presents a great divergence in several gametangia examined.

(1)

A few gametes come to rest in a few minutes after liberation. In 2–3 hours all come to rest, except one gamete. 33 zygotes are formed.

(2)

In one hour a majority of gametes come to rest and 30 zygotes are formed.

(3)

5: 5 gametes are sedentary.
45: A group is found, in which 2 zygotes are formed.
75: 14 zygotes are found; 2–3 gametes are at rest.
300: Resting individuals are not increased.

Also, on the next morning resting individuals are not increased, so that most gametes have been swimming for more than 25 hours. Later, their movement becomes slow and finally ceases.

(4)

180: A group is formed.
220: 2 zygotes are found at the place where the group has been formed.
320: 2 gametes are sedentary.

In this drop the gametangium liberates the gametes at 11.00 a.m. Till 4.30 p.m. only 2 zygotes are formed and 2 sedentary gametes exist, while all the rest are swimming actively. At noon of the next day no additional zygote or sedentary gamete is found. At 2.00 p.m. the active gametes become sluggish and go to rest. Being active so long they look exhausted. Their body assumes an irregular form and becomes slender.

We learn from this experiment that under the same conditions the gametes from different gametangia behave differently, especially as regards the sexuality. Also, among sister gametes the relative length of the swarm period is very divergent.

6. Temperature 18°C.

In a drop of water three gametangia are germinated in succession, and the relation of gametes from one to those from another gametangium is observed (Fig. 7).

The gametangium *x* first liberates gametes.

10: A dense aggregate of sedentary and active gametes is formed at *a*, and there the zygote formation is going on.
15: The member of the group *a* is increasing, while other small groups are being formed at *b* and *c*.
20: At *c* no active gamete is found, a few sedentary gametes and zygotes being present. At *a* and *b* active ones are considerably increased.
21: Two gametangia *y* and *z* germinate almost simultaneously.
25: The new gametes are by a greater part attracted by *a*, and the rest are going to form a new group at *d*.

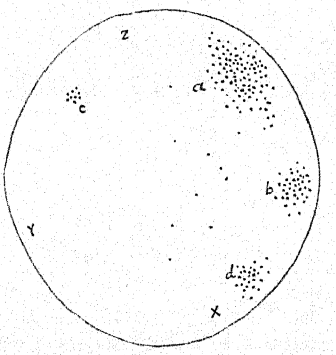


Fig. 7.

30: Freely swimming gametes are very few outside the group *a*, *b*, and *d*.
35: Active gametes mostly swim away from *d*; actives ones are most numerous at *a* and are presenting the fusing action; at *b* many active ones are present. At *a* and *b* a phobotactic movement is clearly exhibited by the swimming gametes. Those swimming away from the group turn their direction and orientate themselves to advance ultimately towards the group.
40: At *d* only one or two active gametes are found; at *a* and *b* the swimming zone of the active ones becomes wider than before, showing, as it were, that the attractive substance existing in the group is diffused.
50: At *a* and *b* active gametes are reduced in number. The swimming zone becomes still wider.
65: The reaction of the active gametes to the sedentary ones is becoming feeble. It appears to show that the sedentary gametes are becoming incapable of exerting an attraction.
85: Gametes remaining still active are swimming about freely in the medium.
100: About 20 gametes are still swimming.
115: Active gametes are further reduced in number; they no more present the reaction towards the sedentary ones.
141: Observation is discontinued.

In the present experiment the process of forming aggregates by gametes is shown very clearly. Active gametes are gathering round the sedentary ones, and some of them sooner or later become sedentary, while the others go to form the zygote. An increase of the sedentary ones in number at the aggregate accelerates the gathering of active ones, making the aggregate larger and larger.

Existence of an attraction zone round the aggregate of sendentary gametes is ascertained in this experiment. Any active gamete once entering this zone is not able to get off and in this manner numerous gametes are deposited there as zygotes or azygotes.

7. Temperature 17°C.

Germination takes place after the gametangium remains for 24 hours in a drop of water.

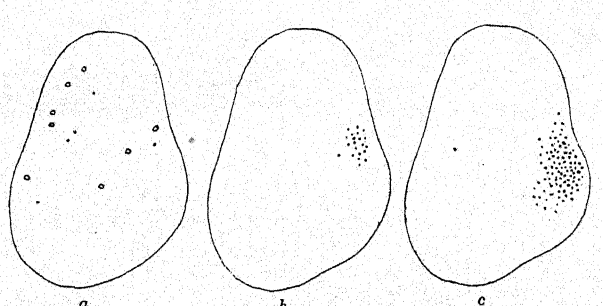


Fig. 8.

10: 8 zygotes and 5 sedentary gametes are found scattered in the medium; active ones are swimming about uniformly distributed (Fig. 8, *a*).
15: The sedentary ones remain solitary.
30: A group of gametes appears, consisting of many sedentary and active ones (Fig. 8, *b*).
35: Most active gametes join the group and those wandering about freely in the medium are very few.
40: In the group swimming individuals become less in number, while sedentary ones are increased.
55: In the group 18 zygotes are found; active gametes that react to the sedentary ones are very few (Fig. 8, *c*).
70: In the medium active gametes, being considerably reduced in number, are wandering about freely, without presenting the fusing action upon the sedentary ones.
80: The zygote amounts to 26 in total.
140: Except 7–8 individuals all gametes become sedentary.

In this experiment it is shown that the gametes coming to rest at an earlier period can easily form the zygote without resulting in the formation of aggregate. The active gametes attracted by them do not stay there if unsuccessful of fusion. However, those attracted by lately sedentary gametes are disposed to deposit while engaging in fusion and they attract in turn the active gametes.

8. Temperature 12°C.

5: Gametes are all actively swimming.
55: All swimming.
75: All swimming.
85: At a spot *a* 4 gametes are at rest (Fig. 9, *a*). Many active ones are crossing it, making a short stop.
95: A zygote is formed at *a*. At *b* a gamete comes to rest, to which passing active gametes show a reaction for a while.

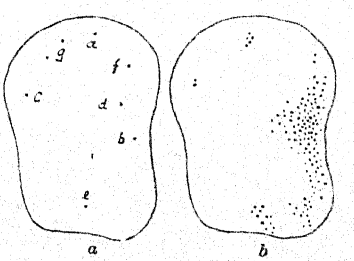


Fig. 9.

98: At *c* a sedentary gamete is found. It soon gives rise to a small group and becomes a zygote.
100: At *c* 2 zygotes and 2 sedentary gametes accompanied by an active one are found. At *a* active gametes are staying.
115: Active gametes are observed to stay for a while at *a*, *b*, and *c*, where sedentary gametes are present.
135: At *d* a group is formed by a sedentary and 2–3 active gametes. At *b* one zygote is formed. At *e* a sedentary gamete appears. At *a* the reaction of active gametes to the sedentary ones is very feeble.
145: At *e* no active gamete stays. At *d* gametes are increased in number and 2 zygotes are formed.
150: An active gamete is present at *e*; at *d* no active one exists.
155: The sedentary gamete at *e* forms a zygote.

159: Active gametes are again found at *d* and *a*, but not at *c*.
161: At *b* and *d* active gametes are increased in number, while at *c* and *a* no active one is found.
165: A new small group is formed at *f*. At *b* and *d* the group is growing larger. At *c* and *a* active ones are present.
170: The group *b* consists of a zygote and of 13 sedentary and active gametes. The group *d* consists of 8 members.
175: At *a* one zygote and 4 sedentary gametes are found; there are 14–15 members at *b*, 4 members (2 of them are zygotes) at *c*, 8 members at *d*, and 6 sedentary and some active gametes at *f*.
176: A new group with 6 members is formed at *g*.
180: 2 zygotes are formed at *f*.
185: Here and there the gametes are going to rest and to attract active gametes.
190: Active gametes hitherto swimming about in the medium are going to assemble near *b*, *d*, and *f*.
195: More than 50 per cent. of gametes are at rest chiefly at the places just given and most active gametes are staying there.
205: Active gametes are considerably reduced in number. The zygote amounts to about 13 (see Fig. 9, *b*).

We see that the number of the zygote is much reduced. The same is true in other drops on the same cover glass. It is due to a low temperature to which the gametes are exposed. While thus the low temperature interferes with the formation of the zygote, the result of the experiment definitely shows that the reaction of the active gametes to the sedentary ones, leading to the formation of aggregate, is presented normally.

C. Conclusion

On the basis of observations and experiments given in the foregoing we arrive at the conclusion that the formation of the zygote depends upon internal and external conditions.

1. Internal condition. In all gametes the swimming and sedentary periods are marked. The fundamental condition enabling the copulation of two gametes is that the one is at the sedentary and the other at the swimming period. The sedentary gamete acquires the property to exert a certain stimulus upon the swimming one which is endowed with the property to react to it. By this means the two gametes are brought into association previous to fusion.

What is the most interesting fact which characterizes the sexuality under consideration is that the sexual reaction of the swimming gamete to the sedentary one disappears when it becomes itself sedentary. It occurs that a sedentary gamete is associated with a swimming gamete, in which the sedentary period is approached, and while expressing the

fusing action the latter becomes sedentary and unable to fuse. Owing to an internal condition the length of the swarm period presents a wide range of variations according to individual gametes. Among gametes derived from a single gametangium some come early to rest, while others retain the swimming state one or two hours longer. Some gametangia produce the gametes, a more or less number of which come to rest in succession during a short interval soon after liberation, while the majority swim longer but attain the sedentary period almost simultaneously or within a limit of few minutes.

In consequence, the formation of the zygote is connected with the feature of variation of the swarm period, so far as the sister gametes of a single gametangium are concerned. The combination of sedentary gametes with those, in which the length of the swarm period much exceeds, warrants copulation. This is evidenced by the fact that the gametes coming early to rest easily form the zygote, as there exist numerous gametes having a far longer swarm period. Generally we recognize a critical period, at which a majority of sister gametes become sedentary in rapid succession. At this period their attitude is soon reversed from an attracted to an attractive state and therefore copulation is not insured.

When several gametangia occur to germinate in the same medium, their earlier and later germination gives a crowd of those gametes which come to rest at varying periods, and a combination of two gametes apt to copulate will be obtained more easily than among sister gametes.

The stimulant which the sedentary gametes secrete to attract the swimming ones is accumulated in the medium, when they are increased in number. It lessens the sensibility of the swimming gametes towards the attractive action of the lately sedentary ones. Therefore, the percentage of the zygote may be reduced when the medium becomes older.

In nature numerous gametangia would germinate together in the same medium and their successive germination would be presented in various ways. Further there may exist the gametangia of the short- and long-swimming types in various proportions. Also the number of gametangia occurring together in the medium may be variable. All these conditions give a great effect upon the percentage of the zygote.

2. External condition. Temperature may be taken as a chief factor for the zygote formation. Infraoptimal temperatures make the rate of germination of individual gametangia differ from one another, lessen-

ing an appropriate combination of the gametes for fusion. They act also as interferring with the fusing mechanism of the plasm of the gamete. Supraoptimal temperatures give rise to the formation of sluggish gametes or interfere with their normal behaviours, and consequently with sexual activity. Thus the extraoptimal temperatures prevent directly or indirectly the formation of the zygote. The optimal temperature, on the other hand, enables the gametes to behave themselves normally and renders the rate of the germination of several gametangia approach each other, whereas the gametes may gain advantage in finding the mates apt for fusion.

So far as observed, existence of certain chemical substances in the medium, which disturb the activity of the gametes, is not favourable for copulation. The fresh gametangia, just squeezed out of the sori, germinate soon and at a similar rate, while old ones germinate in a wide range of time, so that the gametes liberated in the same medium from the former kind of gametangia result in the formation of a larger number of the zygote.

In short, the highest percentage of the zygote is obtained when a mass of freshly released gametangia is submitted to germination in fresh and nearly pure water at a temperature of 20°C.

VI. The Parasitic Development of the Fungus

1. Entry of the Fungus

To observe the process of entry of the encysted fungus bodies (zygotes or azygotes) into the host-cell, the gametangia were submitted to germination on the surface of young leaves or stems of the proper host, and after a few hours or a day the inoculated portion of the epidermis, being removed from the subepidermal tissue, was examined under the microscope. More practically, the gametangia were germinated on a piece of epidermis previously removed from the host and stretched on a slide. The fungus bodies were found deposited on the surface of the epidermis, especially along the limiting lines between the epidermal cells. During an interval of few hours they were carefully watched under the microscope, but no change indicating the process of entry was revealed. Consulting, however, different features exhibited by different individuals we assume that the infection may be established through a series of changes described below.

The change which shows the first stage of infection is a refractive appearance of the fungus body and its deformation into an oval or pear-shaped form, sometimes being angular in outline. Among individuals of such a form there are found those which penetrate the outer wall of the epidermal cell and through a fine pore send out a part of the body into the cell cavity in an indefinite form (Fig. 10). After entry they leave nothing behind, but rarely the orange drops can be found left upon the surface of the host-cell (*b*, *d*, *g*). The refractive appearance and deformed shape are retained after entry (*d*, *e*, *f*), but sooner or later the original form and appearance are restored (*g*). At this stage a hyaline appearance with a faint outline makes the presence of the fungus in the host-plasm very obscure in a living material (*h*). A clear view is, however, obtained when the material is fixed in FLEMMING's solution.

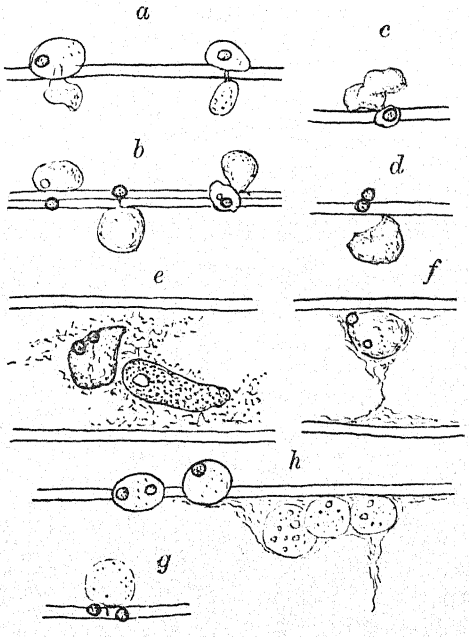


Fig. 10. Several stages showing entry of azygotes or zygotes into the host-cell. Except *e* all are drawn from fresh materials by the aid of a drawing apparatus. *a–c*, fungus bodies in the way of entry; *d–f*, those just entered, yet retaining an angular and refractive appearance; *g*, *h*, a later stage, the body becoming round and hyaline. In *b*, *d*, and *g* orange drops are left behind. (×1200)

Very soon after entry the fungus is increased in size, looking as if swollen on absorbing water. The growth is accompanied by an increase of coarse granules in its plasm.

The refractive and deformed appearance above noted is never assumed by the encysted zygotes or azygotes remaining in the culture medium. The body becomes vacuolate, as it enlarges, and the plasmic content appears as refractive lumps. The orange drops are fragmented or discoloured.

To decide whether the fungus which entered the host-cell is a zygote or azygote is quite impossible. The difference in size is not decisive on this point. The presence of two orange drops may characterize the zygote but soon

after entry the drops seem to be fragmented, becoming indistinctive from the plasmic granules. It is almost certain that at an early part of the growing stage the fungus bodies, either originated from zygotes or azygotes, exhibit the same feature of development.

The number of the fungus bodies to be found in a single host-cell is variable in a wide range. Perhaps there is no limitation about that. In case a large number of the encysted fungus bodies occur on the surface of the host-cell there is a great probability of obtaining a multiple infection under favourable conditions. In fact there are usually five or six in a single host-cell, but often more than that are present, and in an extreme case eleven are found.

2. The Summer Sorus

After infection the fungus proceeds to grow with great rapidity. For instance, in the material inoculated on pot plants in a room it is estimated that an azygote, 5 μ in diameter before entry, attains after that on the average 7 μ on the 2nd, 9 μ on the 3rd, 13 μ on the 4th, 19 μ on the 5th, 40 μ on the 7th, 63 μ on the 11th, and 83 μ on the 15th day.

Until the 4- or 5-day stage the content is colourless, containing a few hyaline granules of unequal sizes. Sometimes at the 3-day stage 2–3 yellowish granules appear as an indication of the generation of the colouring matter. On the 7th day the hyaline granules become larger and are increased in number, being accompanied by an increase of the colouring matter. At about this stage the fungus body is recognized with the aid of a hand lens as a light yellowish spot upon the surface of the host plant. In the living state the nucleus is visible as a hyaline space and a large nucleolus as a refractive globule. Even the other nuclear contents may often be seen distinctly.

At subsequent stages an increase of the granular substances makes the fungus body opaque to transmitted light. Though not clearly, the presence of the primary nucleus can be ascertained still at the 10- or 11-day stage. Division of the primary nucleus is likely to take place at about this stage or a day after. After that repeated nuclear divisions occur and give rise to numerous secondary nuclei.

In individuals of rapid development the segmentation of the body may begin at the 11- or 12-day stage, and in the following 1–2 days the gametangium sorus may attain maturity. Thus the development of the fungus into the summer gametangium sorus may be completed approximately in two weeks.

Here the rate of development is nearly the same as in *Synchytrium endobioticum*, in which, according to WEISS (1925), 10–12 days are required to form the mature gametangia.

Duration required for maturation of the sorus varies according to seasonal conditions, chiefly to temperature. Generally the development is very rapid in June and August, being completed in two weeks. In other seasons it takes a longer time. In spring 20–25 days are required. Inoculated on November 1, 1926, the fungus developed very slowly and its 15-day stage corresponded to the 6- or 7-day stage in summer time. The maturation of the sori took place on December 22, that is, after 50 days. Inoculation upon seedlings on December 12, 1926 in a room (10°–17°C.) yielded nearly matured gametangia after 47 days.

3. The Resting Cell

a. THE DAILY COURSE

Until a few-day stage after infection all the fungus bodies follow the same course of development, and no exact distinction can be drawn between individuals developing into the resting cells and those becoming the summer sori. Soon after, the difference becomes obvious in size and colour. While the individuals for the summer sori are larger and yellow-coloured, those developing into the resting cells are smaller and remain hyaline, containing globular contents. The resting cells are later characterized by giving a milky colour on reflected light. Further, while the fungus bodies for the summer sori grow to fill up the cavity of the host-cell, those for the resting cells leave much space in the cavity, so that at the end of growth their spherical bodies lie freely in the cavity.

When full grown there appears a hyaline membrane round the body as a primordial wall. It rapidly thickens and with the addition of other membranes on its inner and outer surface the wall of the resting cell is completed.

b. THE WALL

The wall of an adult resting cell consists of three layers (epispore, mesospore, and endospore), of which the outer two layers are thick and distinct from each other, while the innermost one is not only very thin but hardly distinguishable from the middle layer.

The epispore is orange brown to dark brown. Upon its surface brown disorganized contents of the host-cell are deposited, on account of which the resting cell acquires a warted appearance.

The mesospore is hyaline, usually thicker than the epispore. By careful observations two layers are distinguished in it, the outer thinner and inner thicker ones. The outer layer is faintly coloured and appears more compact than the inner.

The endospore is very thin and colourless. In a fresh material *in toto* it is not clearly distinguished from the mesospore.

In the material fixed in FLEMMING's solution, the character of the different layers of the wall is exhibited more distinctly than in the fresh one. Pressing the resting cell between a slide and a cover glass, the epispore is split and the mesospore is exposed. Its outer layer shows an appearance of a fibrous structure and the inner one breaks like a gelatinous layer. Meanwhile the endospore remains intact in a sharp contrast to the mesospore. Distinction between the mesospore and the endospore may also be drawn from a change of colour by the fixation; the mesospore takes on a faint colour, while the endospore is colourless. Pressing the resting cell more strongly the mesospore is also split and the endospore is exposed as a thin but distinct hyaline membrane.

Treated with potassium hydroxide the epispore and the outer layer of the mesospore remain intact, but the inner layer of the mesospore is considerably swollen and appears as if gelatinized. Chloral hydrate gives also a similar effect.

Development of the wall: At the growing stage the resting cell is naked, and its peripheral portion is hyaline, being delimited by the plasmic membrane. By the time the fungus is nearly full grown a thin hyaline membrane is formed. At this stage the cytoplasm of the host-cell is accumulated over the surface of the fungus (Fig. 11, *A*). The initial membrane of the fungus proceeds to thicken and develops into the mesospore. The cytoplasm of the host-cell loses its normal structure and is disintegrated into coarse globules of variable sizes (*B*). These globules are deposited on the surface of the mesospore. At first a clear narrow space is visible between the heap of globules and the membrane. The space seems to be represented by a layer of mucilaginous substance secreted by the fungus (*C*). Being sharply demarcated from the globular heap and becoming more compact, it finally constitutes a thin outer layer of the mesospore (*D*). After this layer is distinctly formed, another mucilaginous layer appears over it. In the mean time the content of the host-cell begins to turn brown and

the globular heap becomes denser and compact as if the globules are fragmented. The heap is partly dissolved and the dissolution product impregnates the mucilaginous layer to form a membrane of homogeneous consistence with a faintly brown shade (*E*).

The dissolution proceeds from the lower to the upper layer of the heap, stopping at a definite thickness (*F*). When this action is over, the outline of the homogeneous layer becomes distinct and sharp, and thus the epispore is completed (*G*). The undissolved remains of the globules adhere firmly to the surface of the epispore and finally coagulate into a layer of coarser structure and ununiform thickness (*H*).

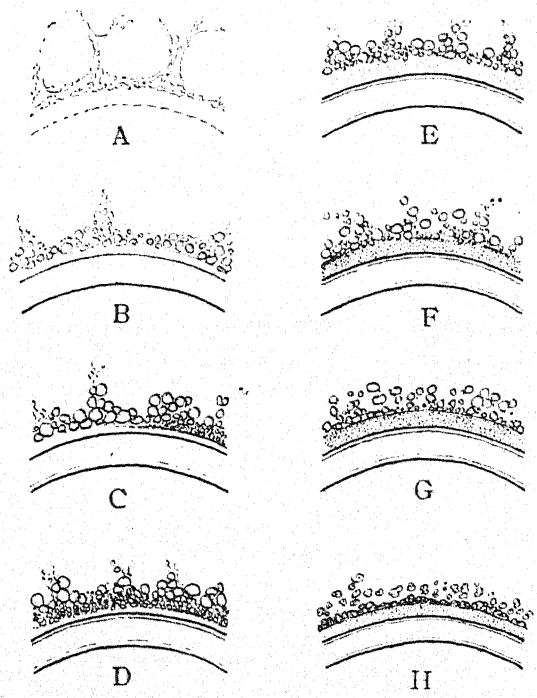


Fig. 11. Development of the wall in the resting cell (from fresh materials).
A, initial of the mesospore; *B*, accumulation of the contents of the hostcell over the thickened mesospore; *C*, globular heap on the surface of the mesospore; *D*, dissolution of the globular heap; *E*, dissolved layer of the globular heap; *F*, the layer becoming the epispore; *G*, epispore sharply demarcated from the rest of the undissolved glogular heap; *H*, completion of the wall. (×ca. 800)

The mesospore is at first thick but by the time the epispore is completed becomes thinner and compact, perhaps owing to withdrawal of water. As to the formation of the endospore the exact period of appearance is not determined. Perhaps it is developed after the completion of the mesospore.

In *S. endobioticum* the origin of the epispore is given in detail by CURTIS (1921). According to her, the contents of the host-cell and its wall contribute altogether to the formation of the epispore. Regarding the precipitation of globular substances (oily matter according to her) upon the mesospore my observation accords

with her's but in our fungus only a part of the contents of the host-cell participates in forming the epispore.

The wall and residual globules of the host-cell become dark brown when the resting cell matures. With this modification the host-cell may withstand the putrefying action of microorganisms. For instance, subjecting a diseased portion of the host plant to corruption in water, the host-cells are freely isolated as intact envelopes of the resting cells. The wall of the host-cells thus freed looks like an outermost membrane of the fungus body, in particular when the cavity of the host-cell is almost entirely occupied by the fungus. Very probably the wall may protect in some way the resting cell inside, but it does not represent any part of the fungus.

With the completion of the wall the resting cell may be said to have reached maturity. Then the activity of the host-cell is ended, though appearing as fresh as the surrounding healthy cells. The inclosed resting cell also remains fresh.[1] When the diseased portion of the host perishes and is dried, the resting cell enters the dormant period on desiccation. A dense accumulation of globules in its cytoplasm gives the cell an opaque appearance. The nuclear cavity is recognized as a large hyaline space, in which a prominent nucleolus and other elements may often be clearly visible.

4. The Genetic Connection between the Zygote and the Resting Cell

From the analogy with *Olpidium Viciae* and *Synchytrium endobioticum* it is beyond doubt that the resting cell originates from the zygote. In the case of *Olpidium* (KUSANO, 1912) the course of development of the zygote into the resting cell can be clearly followed out on account of the binucleate character throughout. In the present case the zygote is uninucleate at the stage of infection and does not present a distinct morphological difference from the azygote. Hence, the genetic connection between the zygote and the resting cell cannot be proved by direct observations. As a most reliable evidence, it may

(1) At the end of November, 1927, summer sori on diseased leaves were found at dehiscence. On the same leaves numerous resting cells have reached maturity. The leaves remained alive and fresh throughout the winter in a laboratory room until April of the next year, and the resting cells upon them were in a fresh and undried condition during a period of 5 months.

be mentioned that the temperature condition which induces the formation of the zygote gives rise to the formation of the resting cell.

As stated before, the gametes from the recently matured gametangia produce the zygotes in larger percentage at a temperature of 20°C. On applying the similar gametangia to the host plant at the same temperature a predominant formation of the resting cell takes place. On the other hand, the inoculation made at temperatures, at which the formation of the zygote is less, yields always a small percentage of the resting cell, its formation being greatly restrained at a temperature approaching the maximum or minimum for the zygote formation.(1)

Thus we find a coincidence of an optimal temperature for the zygote formation and for the occurrence of the resting cell. From this fact the genetic connection between the zygote and the resting cell is almost evident.

This statement is supported by the field observations. In the field the amount of the resting cell presents a seasonal fluctuation; less in the early spring, increasing towards summer, very small during summer, again increasing towards autumn, and decreasing in approaching winter. Such a fluctuation may largely depend upon a seasonal change

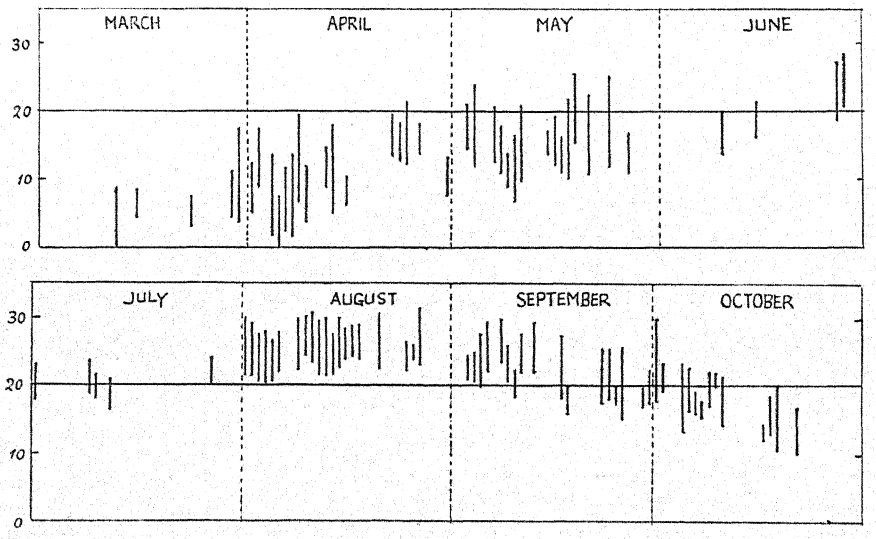


Fig. 12. Temperature ranges on rainy days in 1925 (from the record of the Tokyo Meteorological Observatory).

(1) See the infection experiments to be given in a later section.

of temperature. Meteorological observations show that the temperature condition in the late spring and autumn is favourable for the formation of the zygote and consequently for the resting cell. In rainy days, in which infection is enabled, the temperature in an early period of spring is usually far below the optimum (20°C.) for the zygote formation. As the climate is progressively getting warmer, the temperature in rainy days is so much increased as to fluctuate above and below the optimum, inducing the gametes to copulate. Entering summer the lowest temperature on rainy days lies above 20°C. Again, the autumnal climate presents a temperature condition similar to that in the late spring. However, on approaching winter the temperature falls to a degree rendering the gametes difficult of copulation. The course of the changes of daily temperatures on rainy days during the vegetative season of the year 1925 is given in the accompanying figure (Fig. 12).

5. Competition between Fungus Individuals in the Same Host-Cell

We have already noticed that the zygote or azygote may invade a single host-cell in varying number, multiple infection being more common than single infection. But, as a fact, only a single summer sorus or a resting cell comes usually to maturity in each host-cell. Two sori or 2–3 resting cells, but not more, may attain to complete development in a host-cell; this is, however, of uncommon occurrence. Evidently, competition for development occurs among individuals invading the same host-cell. A limited supply of nutritive substances and also a limited room for rapidly enlarging fungus bodies would perhaps restrict the number of individuals that attain a complete development.

As showing the competition an individual difference in size is already presented on 2–3 days after infection, subsequently becoming more remarkable. This difference at such an early stage might possibly be brought about by the different nature of the individuals, those which develop into the summer sori being more rapid in growth than those which become the resting cells, or else it is due to different times of infection. At any rate it seems certain that the largest one is the strongest and is able to survive, while the other individuals perish at various stages during their development.

On considering the competition the occurrence of two mature sori in the same host-cell is very interesting. Generally both sori are almost equal-sized, being each hemispherical but together ellipsoidal in form. On surface view they give an appearance of a single sorus, but usually they dehisce at different periods, so that the occurrence of two sori is made apparent. As to the development of two sori of an equal size we conceive a balance of competition between them and a similar degree of their vigour, with which they compete with the remaining coexistent individuals.

In the case of the resting cell the occurrence of more than one in a host-cell is more frequent than in that of the sorus. It can be explained by assuming that the competition is taking place moderately. The host-cell, though not enlarged so much as that of the sorus, can furnish a sufficient room for residence of 2–3 resting cells. It can also supply nutritive substances sufficient for more than one, as the resting cell devours less than the sorus.

The above consideration is related to the competition as prevailing between either the sori or the resting cells themselves. However, as might be expected, zygotes and azygotes together may invade the same host-cell. A question naturally arises as to what occurs in this case of multiple infection. The field observations and the results of inoculation experiments bring out the fact that the summer sorus and the resting cell occur as a rule in different host-cells. As a natural consequence, we induce that a competition must have prevailed between the azygote and the zygote during the course of their parasitic development in their common host-cell, either the sorus or the resting cell being developed as the survivor.

In explaining the said relation between the sorus and the resting cell, experimental evidences are not at hand. Yet, the following observational facts may appear to help in elucidating the matter concerned.

When both summer sori and resting cells are formed in a diseased patch, they exhibit a characteristic arrangement. If infection is not severe, they are arranged intermingled throughout the patch, sometimes a few summer sori among numerous resting cells, and *vice versa*, according to the conditions under which the formation of the zygote is controlled. This feature presents no peculiarity, as it seems quite natural when both the zygote and the azygote invade independently the different cells (Figs. 13, *a*; 14). As an outstanding feature when very severe infection is obtained, the summer sori occupy the marginal

portion of the patch which is produced almost entirely by a vast number of the resting cells (Figs. 13, *b*; 15). It may be true that in producing such a patch the zygote formation has been prosperous there. Yet, on the basis of germination experiments it is evident that a more or less number of azygotes would have been present mingled together.

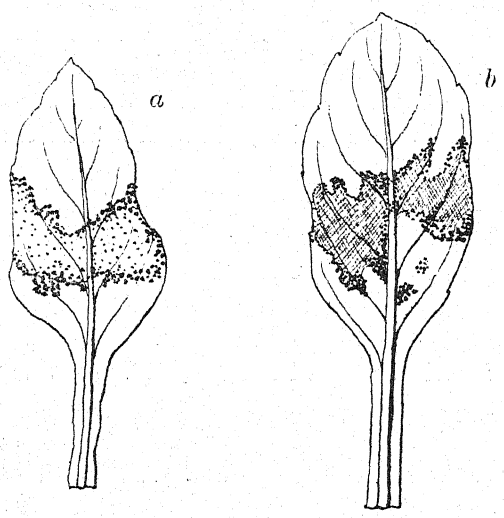


Fig. 13. Distribution of the summer sori on the diseased patch produced by inoculation in autumn (dots represent the summer sori and shady lines the area occupied by the resting cells). *a*, summer sori uniformly scattered (infection slight); *b*, summer sori exclusively peripheral in position (infection severe). (Nat. size)

Regarding the absence of the summer sori within the patch we cannot conceive the prevalence of any condition that interferes with the infection by azygotes, since their invasion is shown by the occurrence of the sori along the margin of the same patch. Therefore, no other explanation is allowable than that the azygotes actually enter the epidermal cells lying within the patch, but perish during their parasitic development. When we examine a patch, upon which the summer sori are uniformly distributed, we find that their development is most vigorous on its marginal portion, since the nutritive condition of the host-cell is, in my opinion, most favourable there (Fig. 14). Also, as already pointed out, the development of the summer sori, unlike that of the resting cells, requires a great activity of the host-cell. For these reasons we take it as very probable that within the patch the supply of nutritive substances by the host-cell is insufficient for the devouring azygotes, though it may be sufficient enough to sustain the development of zygotes into the resting cells. Along its margin, however, the nutritive condition of the host-cell enables the azygotes to develop into the summer sori, and their vigorous growth prevents the development of the zygotes occurring in association.

If the infection is not severe, an association of azygotes and zygotes in the same host-cell will occur less freqnently. On the one

hand deficiency of nutritive substances in each host-cell is not so great as in the case of severe infection, and on the other there may occur

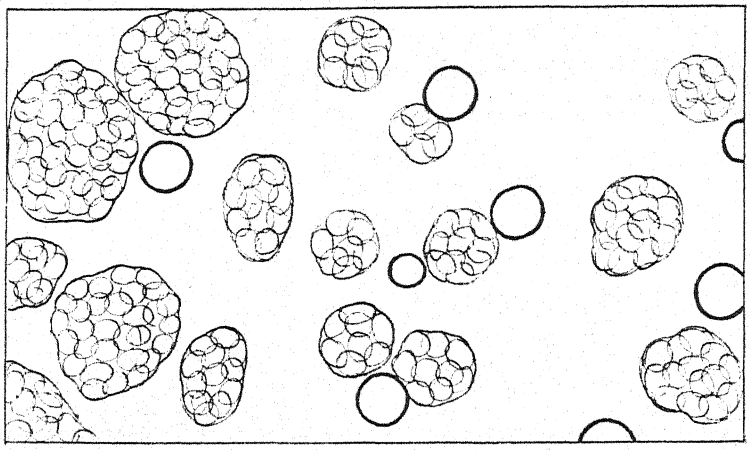


Fig. 14. Different sizes of the summer sori on the periphery (left) and the inner portion (right) of the patch (from the leaf given in Fig. 13, *a*). (×150)

those azygotes which are not associated with zygotes and can secure the complete development into the sori (Fig. 14).

In the case of severe infection we rarely find both the mature summer sorus and resting cell in a host-cell that lies near the periphery of the diseased patch (Fig. 16, *d*). This fact is very interesting as it amplifies the relation between the nutritive condition of the host-cell and the competition. At early stages of the fungus development the coexistence of growing azygotes and zygotes in the same host-cell is ascertained throughout the extent of the patch, but the occurrence of the mature sorus and resting cell in association is limited to the portion just mentioned. Obviously the nutritive condition of the host-cell is decisive for giving rise either to the mature sorus or resting cell, or to both. As has been stated before,

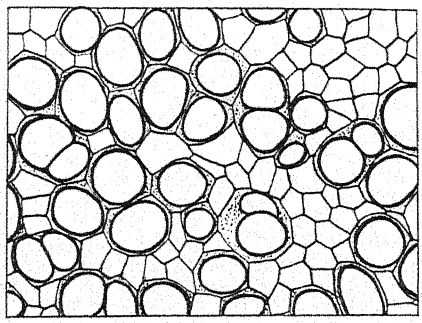


Fig. 15. The central portion of a heavily infected patch, being exclusively occupied by the resting cells (from the leaf given in Fig. 13, *b*). (×150)

on the margin of the patch it induces suppression of the resting cell (Fig. 16, *a*) but in the central portion its survival (Fig. 16, *b*, *c*). Between these extreme nutritive conditions an intermediate one prevails in a peripheral portion of the patch. Under this nutritive condition a balance of competition may be maintained, enabling both the azygote and the zygote to complete the development.

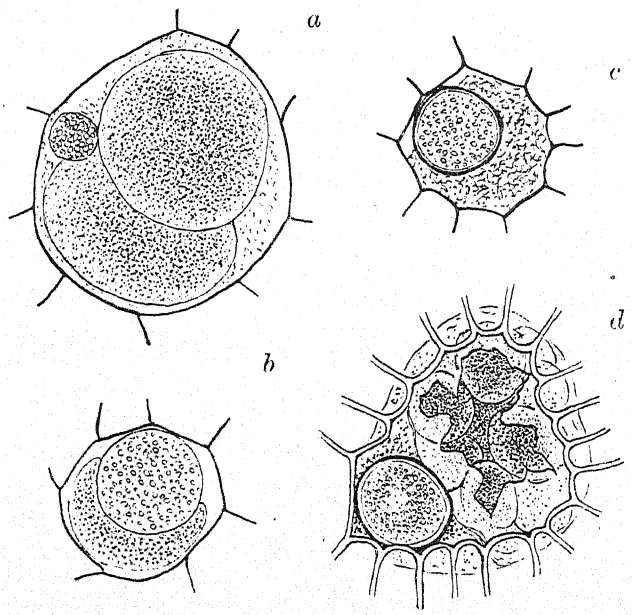


Fig. 16. Competition for development between the summer sorus and resting cell on different portions of a diseased patch. *a*, marginal portion, two vigorous prosori and a resting cell stunted in growth; *b*, inner portion, a young resting cell and a compressed prosorus; *c*, the same portion, a mature resting cell and a disorganized prosorus; *d*, a portion between *a* and *b*, both the sorus and the resting cell have attained maturity. (×300)

Now we come to the following statement as regards the relative number of the summer sorus and resting cell to be developed at different seasons:

At an early period of the vegetative season, when the host plant is vigorously growing, the zygote may be suppressed in development by the azygote coexisting in the same host-cell. This results in the predominance of the summer sori at that period. On the other hand, at a later period, when the host is stunted in growth, a reversal of the

suppression may occur between the zygote and azygote, leading to the predominant formation of the resting cells.

Thus the relative number of the summer sorus and resting cell is controlled primarily by the temperature acting as a decisive factor for the zygote formation, and secondarily by the nutritive condition of the host plant or the host-cell acting so as to cause competition between the azygote and the zygote happening to develop in the same host-cell.

VII. The Infection Experiments

To furnish sufficient materials for the present work inoculation experiments have been extensively made. Some of the results have already been referred to. In this place it is proposed to record some of the experiments which may be helpful in explaining the facts obtained from the field observations regarding the life-history of the fungus.

For inoculation potted rosette plants of *Oenothera Lamarckiana* or *O. biennis* were generally used. Also cut shoots were used, as they were more practical for this purpose. They were taken about 20 cm. long, and, except a cluster of young leaves on their apices, all the other leaves were removed. Standing in water vessels the shoots remained for a long time in a fresh condition, producing new leaves on the growing top and sometimes adventitious roots in water. As the inoculum small pieces of affected leaves, on which summer sori were just dehiscing, were applied to the host, and a mass of cotton fibres soaked with water was laid over them. Sometimes gametangia were gathered upon a mass of cotton fibres and the cotton mass, being directly applied to the host, was saturated with water. After inoculation the host plant was kept under a bell jar till the next day. During that interval the gametangia began to germinate and the infection soon took place.

1. Inoculation of the Host at Different Stages of Development with the Summer Gametangium

1. Inoculation of bud-leaves: In the field the leaves forming a certain whirl or whirls of the rosette are found equally affected and the fungus individuals upon them all show a similar stage of development. They seem to have attacked the leaves at the same time. A similar feature of infection was revealed in the inoculation experiments.

When the gametangia were applied to the center of the rosette or to the top of the shoot, a number of small bud-leaves were simultaneously infected. After they grew up, arranged in whirls, their infected areas were considerably expanded and became very conspicuous.

In the case of heavy infection the orientation of these areas was very characteristic. Being often sharply delimited, they occupied the basal, middle (or whole), and apical portions of the leaves in the outer, middle, and inner whirls respectively, as has frequently been noticed in the field observation (Fig. 17). From this experiment we see that the natural infection in the field may proceed as follows. The gametangia on the diseased leaves are carried by rain water down to the cluster of bud-leaves. For several hours after rainfall the liquid water may persist there affording a medium for the germination of the gametangia. In the bud-leaves the outer larger ones would have only their lower portion immersed in the medium, while the inner ones are entirely dipped into it. It is natural to consider that the infection extends on the bud-leaves as far as the area covered by the medium water. After infection is over, the affected leaves grow larger and show the orientation of the diseased portion as given above. As the lower portion of the affected leaves in the inner whirl is developed after infection, it remains free from the fungus (Fig. 17, *a*).

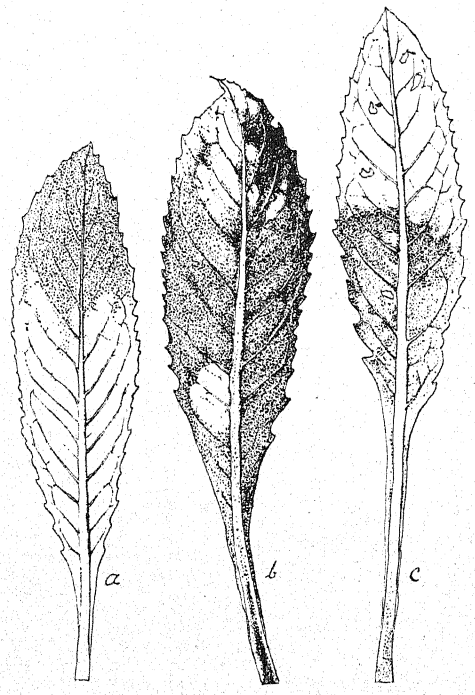


Fig. 17. Distribution of the diseased patch on leaves in inner (*a*), middle (*b*), and outer (*c*) whirls of an affected rosette plant. (×1/2)

2. Inoculation of old leaves: While the infection is found as a rule on young leaves during the vegetative season, the first outbreak of the disease in spring on the over-wintering rosette plant takes place on the outermost, oldest leaves. To test the infection of adult leaves inoculation was made on some leaves of a flowering shoot, which were

full grown and did not show any further increase in dimension. The infection took place very easily and the summer sori there developed attained the normal size as could be seen on young leaves.

As a remarkable fact the epidermal cell having once attained an adult stage began again to enlarge when the fungus was beginning to grow therein. Also, as might be seen in young leaves, the cells surrounding the host-cell regained their activity and underwent multiplication.

3. Inoculation of seedlings: In the field very young seedlings were often found affected. To ascertain the feature of infection in this case the seeds of *Oenothera biennis* were sown in the PETRI's dish. In a week or two they sent out long radicles. Fresh gametangia were dusted over them. After a few days a number of gametangia have germinated and numerous encysted gametes or zygotes were found resting on roots, root-hairs, cotyledons, and hypocotyles. After some weeks yellow spots often appeared densely on the cotyledons and hypocotyles, showing a good infection. However, in the epidermal cells of the root no individual of the fungus was detected. In the anatomical character of the epidermal cell it is almost impossible to draw a distinction between the hypocotyle and the root. Yet, the occurrence of the fungus may often aid us to determine the lower limit of the hypocotyle (KUSANO, 1929 b).

2. Inoculation with the Summer Gametangium at Different Temperatures

Assuming that the zygote develops into the resting cell we expect a yield of the resting cell when inoculation is made at the temperature optimal for the zygote formation. Bearing this in mind the experiments were carried out at different temperatures.

1. (August, 1925). Clusters of young leaves were inoculated with summer gametangia, one set at 25°–26°C. and the other at 17°–18°C. On the 13th day the infected portion became very apparent upon the grown leaves, and summer sori or resting cells could be clearly detected. In the set inoculated at the higher temperatures the infection was slight, and the summer sori predominated, the resting cells being found here and there among the sori. The set inoculated at the lower temperatures obtained a better infection. The summer sori were fewer than the resting cells, and in fact some of the diseased patches were almost exclusively occupied by the resting cells.

2. (August, 1925). Inoculation was made at 26°–27°C. on one set, and at 17°C. on the other. At the higher temperatures the infection was very slight, producing chiefly the summer sori, though the resting cells were not entirely exempt. At 17°C., however, a heavy infection resulted, and large diseased patches appeared on several leaves. They were chiefly occupied by the resting cells, the summer sori lying along the margin of the patches.

3. (August, 1925). Inoculation was made at a wider range of temperature.

14°C.—Good infection; almost no resting cell.

18°C.—Heavy infection; the resting cells predominated.

25°C.—Diseased spots appeared; the presence of the resting cell obscure.

27°C.—Infection unsuccessful.

From these experiments it will be seen that the inoculation at a temperature which is near to the optimum for the germination of the gametangia and for the presentation of the normal behaviour of the gametes yields a good infection, producing the resting cell in preponderance. Above and below that temperature, not only is the infection less successful but the formation of the resting cell is largely inhibited. The most favourable temperature for producing the resting cell coincides exactly with that for the formation of the zygote. This fact is an evidence for the genetic connection between the zygote and the resting cell.

3. Inoculation with Winter Gametangium

The zygote formation by the gametes from the winter gametangium has already been ascertained. This fact makes it almost certain that the generation of the resting cell derives the generation of both the summer sorus and the resting cell. We proceed now to confirm it by inoculation experiments.

1. (April, 1925). The winter gametangia which remained ungerminated in the germinating medium of the resting cells were inoculated on young hosts at a room temperature. After 40 days dehiscing summer sori were obtained. Among them the occurrence of the resting cells was ascertained with certainty.

2. (November, 1925). The winter gametangia were brought to germination in a hanging drop at 22°C. On the next day numerous

gametes and zygotes were found already encysted. The drop was then applied to a young portion of the host grown in a room under a bell jar. Infection went on fairly well and both summer sori and resting cells were produced in the same diseased patch.

3. (April, 1928). A pot plant over-wintered in a room was inoculated with the germinating resting cells. Dense infection has taken place and large distinct patches were produced on the surface of inoculated leaves. Remarkably, the summer sori occupied the peripheral portion of the patch, while the inner portion was densely covered by the resting cells.

4. (January, 1926). Winter gametangia were applied to a bundle of seedlings of *Oenothera Lamarckiana*, and the bundle was kept at 21°C. for two days. Infection took place on all portions of the seedlings, except the root, and the development of the resting cell was decidedly ascertained.

Thus we see that the result of inoculation with the winter gametangium is quite similar to that with the summer gametangium as already given.

4. Soil Infection by the Resting Cell

1. (March, 1926). Rosette forms of *Oenothera biennis* which had over-wintered outdoors had been spreading the outermost living leaves close to the surface of the soil. Pieces of affected stems, which were kept underground, and upon which mature resting cells were found in great abundance, were laid beneath these leaves. Care was taken to keep the pieces and the soil sufficiently moist. The temperature presented a wide change between day and night. Observed after a month the under surface of some of the leaves, with which the affected stems were in direct contact, was found to carry a number of summer sori. The temperature condition at this period of spring was not yet favourable for the germination of the resting cell and the gametangium, and also for the copulation of the gametes. Consequently, the infection was very slight and the resting cell was not produced.

2. (September, 1925). The seeds of the host plant were sown in pots in August. In September they germinated and cotyledons made their appearance above the surface of the soil. At this time pieces of the diseased dead stems of the host, which had been attacked at an early period of the season and had been bearing a vast number of the mature resting cell, were laid over the seedlings. During October frequent rainfall rendered the soil condition favourable for the germi-

nation of the resting cells and of the derived gametangia. At the end of that month cotyledons, hypocotyles, and young leaves were found furiously affected. The summer sori were produced in predominance, while the resting cells were very few.

In this experiment we see that the resting cell may germinate in the field in the year of formation and may produce the disease upon seedlings by soil infection.

3. In the autumn of 1927 the seeds of *Oenothera biennis* were sown in a small bed prepared with soil contaminated with abundant resting cells. In April of the next year numerous seedlings appeared which were almost all affected by the fungus. Not only the cotyledons and hypocotyles but also the first 2–3 foliage leaves were attacked; probably the infection had taken place while the seedlings were lying beneath the surface of the soil.

VIII. Discussion

It is not intended in this place to make a general survey of all problems treated in the present investigation, but only the subject of the sexuality will be dealt with.

1. Sex Differentiation[1]

In considering the mode of sex differentiation in the present case of isogamy our attention should be, first of all, called to the fact that the fungus presents haplomonoecism. A single gamete develops into the summer sorus. The sorus is haplophase, so that all gametes derived from a single sorus should genotypically be similar to each other and to the gamete from which they are descended. Therefore, as to the copulation between these gametes the genotypic sex differentiation is out of consideration.

As an interesting fact, the morphologically and genotypically similar gametes show at the time of copulation a distinction between male and female by their being respectively swimming and sedentary. However, it should not be understood that the swimming gametes are invariably the male and the sedentary ones the female individuals. Since all gametes are first swimming and finally sedentary, it will be seen that the sex character is not constant in each individual gamete, being male in an early and female in a later life stage.

(1) A preliminary note on this subject has already been published (KUSANO, 1928).

During the whole life of the gamete, as in that of an individual organism, we may conceive a succession of changes in internal physiological conditions. The appearance of the sex character seems to be correlated with these conditions.

On this assumption I propose to represent the whole aspect of the sexual phase in a gamete by an accompanying graph (Fig. 18).

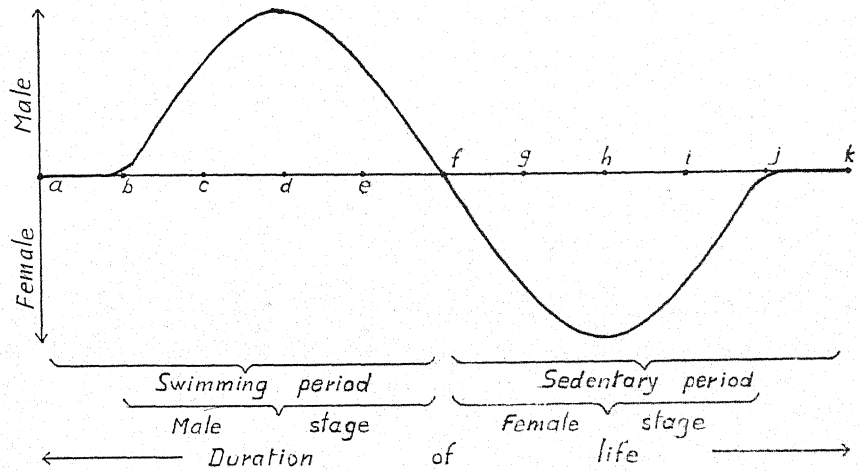


Fig. 18. Graph showing the alteration of sex in a gamete. Explanation in text.

a and *k* represent respectively the beginning and end of the life, that is, the time of liberation and encystment. At a certain period of this life duration the male character is displayed (*b*), which is becoming stronger till a climax is attained (*d*), and then it tends to decrease till it vanishes at about the time when the sedentary period is reached (*f*). The female character is then developed and follows a course (*g*, *h*, *i*) similar to that of the male character but vanishes previous to encystment (*j*).

We now proceed to consider how far this representation can be justified by the facts gained from the present investigations.

(a) For a short length of time after liberation the swimming gamete is in its highest activity and is quite indifferent to copulation with the coexisting sedentary one. It may be due to the fact that the male character is not yet developed (*a*–*b*).

(b) When gametes meet with the female, some show a feeble reaction for fusion, as may be expressed by their flying away after a

trial for a short interval, while the others stay longer. An acquisition of the male character is still feeble (*b–c*).

(c) Irrespective of success or non-success of copulation, some males stay longer by a female, presenting a great endeavour for fusion. The male character attains a climax (*d*).

(d) Swimming gametes, while engaging in copulation, become feeble in movement and after a while come to rest, being sometimes copulated by another swimming one. They are in a transitional stage from the swimming to the sedentary period, so that at the time of coming together with the female the male character has been decreasing (*e–f*) and is to be soon succeeded by the female character (*f–g*).

(e) Generally, the gametes becoming sedentary are not reacted upon for some minutes by the males passing closely to and fro. Perhaps the female character is at the beginning of development (*f–g*).

(f) Females attract a number of males but no copulation takes place. They approach the encysting stage, so that the female character is becoming feeble (*i–j*).

(g) Females formerly attracting male gametes become afterwards solitary. They attain the encysting stage (*k*).

Thus several mutual reactions presented by gametes concerned in sexuality can be explained by the assumption of the sex alteration. Therefore, we may state that the sex differentiation in gametes is expressed, *not in terms of individuals, but in terms of the life stages.*

In this mode of the sex differentiation we recognize individual variations among gametes. The most apparent variation is the duration of the male stage which can be measured almost exactly by the length of the swarm period. As already given, some gametes become sedentary in a few minutes after liberation, functioning then as females. The duration of swimming is here extremely short. It is not evident whether they have been the males previous to becoming the females. Probably the male stage may be practically omitted. There are also a number of gametes retaining the swimming activity for several hours, while the others have already attained the sedentary period. The retention of the male character during that interval is evidenced by their reaction to the coexisting females. However variable the length of the male stage may be, that of the female stage is nearly constant and short. So far as observed, an expression of the female character in sedentary gametes does not on the average last longer than 20 minutes.

Further, it appears probable that an individual variation is possible regarding the degree of sex and the period of appearance and disappearance of the sex character in relation to the swimming and sedentary periods. If this be true, we would expect the presence of the weak female or male gametes, and an earlier or later appearance of the female character upon the sedentary gamete. However, we are not as yet in a position to give conclusive evidence for it.

Alteration of sex during the life stage is known in animals, taking place in an individual body. In an intersex (GOLDSCHMIDT, 1923, p. 91) an individual develops as a male (or female) up to a certain life stage; then the development continues as a female (or male). What is termed consecutive monoecism included in the category of hermaphroditism presents a much closer resemblance. Here an individual is first male, then becomes female (GOLDSCHMIDT, 1923, p. 191). In *Crepidula*, a genus of Mollusca, the male is free-swimming, while the later female phase is displayed when sedentary, a feature quite similar to that exhibited by the gamete of the fungus under consideration. I may say that consecutive monoecism is an appropriate term to express the sexual nature of the gamete.

In the present state of our knowledge on the sex differentiation it is generally accepted that the sexual cell possesses the fundaments ("Potenzen") of both sexes and an appearance of a male or female character is ascribed to the unfolding of one of the fundaments. In the present case both fundaments are unfolded in each gamete but at different life stages.

We may now come to the consideration of internal or physiological conditions for the consecutive appearance of both sex characters. In a series of physiological processes throughout the life of the gamete we may distinguish two main phases, the first phase being marked by the swimming and the second by the sedentary period. As a fact the male and female characters appear in connection with each of both phases. It can be considered that the individual variation in the length of the swarm period is an expression of the individual variation in duration of the physiological processes in the first phase; in other words, the physiological conditions are similar in all gametes while they are at the swimming period, whether that period be long or short. Perhaps unfolding of the male fundament may have some connection with these conditions. It is emphasized that the turning points both of the physiological phase and of the sex character always coincide with each other in each gamete.

Considered physiologically, the sexual difference between the copulating gametes is perceived by the attraction of one and reaction of the other to it. We conceive that whenever the gamete becomes sedentary, its physiological condition is so changed as to exert a certain stimulus upon the swimming one. Assuming the stimulus to be due to a certain chemical substance, it may be generated, or accumulated to a certain amount, as a result of metabolism in the body of each gamete, now becoming immovable; in other words, it is not produced or accumulated as long as the swimming activity is maintained. Every active gamete has the property of being stimulated by this substance and in turn to generate it after becoming sedentary.

Granting an intimate relation existing between the sex differentiation and the physiological condition, it can scarcely be doubted that external conditions may exert certain effects upon the sexual activity through a change in the physiological conditions. The validity of this notion may be supported by some of experiments already given. Infraoptimal temperatures cause more or less lengthening of the swarm period, that is, shifting of the turning point of the first physiological phase to a later period. Yet, so far as studied, attraction and reaction of the gametes are not interfered with in any way in this case, the aggregate being formed in an usual manner. It shows that the male character is maintained as long as the swimming period is retained, the female character then appearing correspondingly later than usual. If, on the other hand, the period of alteration of the sex character is not simultaneously shifted, so that the female character appears at the usual period, it should happen that the gamete acquires the female character while yet swimming, and at the time it becomes sedentary the female stage is over. This is not in accordance with the experimental results. Existence of certain correlation between the physiological condition and the sex character is almost evident from this fact.

However, as shown by the result of experiments, the fusing action of two associated gametes is considerably interfered with by the said temperatures. Perhaps the fusing mechanism of the plasmic masses of the two gametes undergoes a certain modification through the action of extraoptimal temperatures upon the physiological processes.

On arriving at this conclusion we may dwell upon a special case in the mode of sex differentiation in isogamic lower organisms which

exhibit the relative sexuality. A hypothesis of the relative sexuality was postulated by HARTMANN (1923) a few years ago. The experimental studies carried on in *Ectocarpus siliculosus* by him (1925) and in *Dasycladus clavaeformis* by JOLLOS (1926) led them to arrive at the same conclusion that in the isogametes of the given algae there are individuals which are sexually intermediate between absolutely male and female ones. So that both authors distinguish strong and weak males or females, copulation being possible between strong and weak females (or males).

Comparing now the details of sexual action exhibited by these algae and *Synchytrium fulgens*, a striking resemblance is found. It is based on the following essential points:

(1) Relative copulation.—Gamete B behaves as a male towards A, but as a female towards C. Such a relation is quite similar in *Ectocarpus*, *Dasycladus*, and *Synchytrium*.

(2) Sedentary stage and femaleness.—In *Ectocarpus* the female gamete is as a rule sedentary at the time of copulation. In *Synchytrium* the sedentary state is a necessary condition in one of two gametes for insuring copulation.

(3) Individual variations in the length of the swarm period.—In *Ectocarpus* the length of the swarm period of all gametes derived from the same mother plant is the same and constant, while it is variable according to different mother plants. In *Synchytrium* a single gametangium produces the gametes having variable lengths of the swarm period.

(4) Period of copulation.—In dioecious *Ectocarpus* and *Dasycladus* copulation takes place soon and abundantly, or later and rarely, when the gametes from different mother plants are brought together. In monoecious *Synchytrium* sister gametes copulate at variable periods after liberation, and, as in the algae, copulation is successful at the earlier periods, becoming less frequent at later periods.

From the above it is expected that if the gametes of *Ectocarpus*, which are derived from several mother plants, are liberated together in one medium, their sexual action would reveal features similar to those seen when the sister gametes of *S. fulgens* are liberated in a medium.

In spite of much similarity in the algae and the fungus under consideration as regards sexuality, the conclusion to be arrived at relating to the mode of sex differentiation is fundamentaly different in both cases. In the algae HARTMANN and JOLLOS maintain constancy of the sex and its degree, while in the fungus I maintain inconstancy.

How this divergence is induced is a point for discussion at some length.

In the algae copulation occurs between the gametes from two different mother plants suggesting that the sex differentiation takes place in the individual mother plants, probably phaenotypically (HARTMANN, 1929, p. 11). As an index showing the degree of each sex (weak or strong) HARTMANN has mentioned the length of the swarm period and the feature of copulation presented by the gametes from different mother plants of the same sex. According to him (1925, p. 457), female gametes have a shorter swarm period than that of male ones, and in strong males a longer period is presented than in weaker ones. If the same conception be extended to the case of *Synchytrium*, it would be that the gamete having a short swarm period represents a strong female individual and the one having a long swarm period a strong male individual, while those gametes that have intermediate lengths of the swarm period are weak in femaleness as well as in maleness. This conception may be plausible, but unfortunately it is objected to on the following grounds:—

(a) If each gamete is assumed to have originally developed as a male or female individual, how can the presentation of the sexual action at a definite period of life—the period at which the females become sedentary—be explained? We must infer that the males swimming about together with the females in the medium would remain indifferent to sexuality till the females attain the sedentary period, so to speak, the maturation period. Then it must follow that the female should remain indifferent to sexuality during the previous swimming period. In our fungus this is inconsistent with the fact that almost all swimming gametes react to the sedentary one, and furthermore, the swimming individuals do not copulate with one another.

(b) Granting the existence of strong and weak sexes there may arise a question. Assuming the copulation taking place between a strong and a weak female, the weak one must be said to have displayed the sexual action as a male previous to attaining the sedentary period, and also the same assumption, being extended to the case between a strong and weak male, would induce that the weak one displays the sexual action (as a female) first on coming to the sedentary condition. This inference contradicts the conception of the constancy of the sex character.

(c) Recognition of different sexes among the sister gametes is impossible while they are all at the swimming period. Sexual process

is initiated by the appearance of sedentary individuals, and by their exerting an attractive stimulus upon the swimming ones. The phenomenon of the attraction of the sedentary and the reaction of the swimming gamete to it, which I propose to call a sexual affinity, is a criterion by which sexuality can be judged. Now, on the assumption that there are strong and weak males or females, one may presume that the sexual affinity would be presented in different degrees in different combinations of two gametes, expressing a strong and weak attraction or reaction. In fact, cases indicating a weak affinity are often observed, but, as previously explained, they may be understood on consideration of developmental stages of each sex character during the life of each gamete. On the whole, each gamete acquires, as a male at one time and a female at the other, a critical stage, at which the sexual affinity is presented in a normal degree. In consequence, we may distinguish sexually weak and strong stages in the gamete, but not weak or strong individuals, as far as the sexual affinity is concerned.

If, for instance, a weak female individual exists as assumed by HARTMANN, it should be that it attracts any male and at the same time is attracted by a strong female. Then we must say that the gamete is a weak female and at the same time a male. The gamete of our fungus does not show any behaviour admitting such an inference. In *Ectocarpus*, according to HARTMANN's observations, the so-called weak female is swimming when it goes to copulate with a strong female but is sedentary when copulated by a male. Thus it appears that maleness and femaleness are expressed at different periods. Constancy of a weak femaleness is not perceivable.

(d) Of frequent occurrence is non-success of copulation between the gametes brought to approximation by virtue of the sexual affinity. Copulation may be effected on one to two minutes in one case and on more than 10 minutes in the other. However, it may be unsuccessful, even though the sexual affinity appears very strong and is presented for 15 minutes or longer.

JOLLOS (1926) distinguishes in *Dasycladus* a sexual constitution (" Tendenz ") ,by which copulation is effected, from a sexual affinity, by which the individuals of both sexes are brought together. If this distinction be existent in our fungus, it may appear that the success or non-success of copulation depends upon the strong or weak sexual constitution. This is not, however, in accordance with the fact gained from observations. A sedentary (female) gamete (F) is visited by a

swimming (male) gamete (M_1). While the latter is engaging in copulation another gamete (M_2) comes into association. Copulation may be effected between F and M_2 or between M_1 and M_2. On acceptance of different degrees of constitution the former case is likely to show that M_2 is stronger in maleness towards F than M_1, while the latter case is likely due to the fact that M_1, though it has been a weaker male towards F, behaves towards M_2 as a female, but without doubt weaker than F. Then we come to the conclusion that as regards the sexual constitution M_2 fuses, not with the stronger female F, but with a weaker one, M_1.

Another fact is against the acceptance of correlation between the copulating action and the sexual constitution. As already mentioned the percentage of the zygote is reduced when the gametes are exposed to infra- or supraoptimal temperatures. As a remarkable fact, the sexual affinity is not in this case weakened, as the aggregation of sedentary and swimming gametes has been observed just as at an optimal temperature. As regards the reduced percentage of the zygote it would be absurd to assume that the sexual constitution is changed or weakened at extraoptimal temperatures, while the sexual affinity is not interfered with.(1)

I am inclined to the view that non-success and success of copulation, or its ease or difficulty, may be explained without consideration of the sexual constitution and affinity. Directing our attention towards the fusing mechanism of two gametes, it may be supposed that the two plasmic masses in contact with each other are exerting a reciprocal action, by means of which the physical nature of the plasm undergoes such a modification as enables a simultaneous breaking of the two plasmic membranes. A critical stage of such modification is reached easily and early, or with difficulty and later. Variable duration of the contact of two gametes with each other, irrespective of success or non-success of fusion, would support this assumption. Moreover, a reduction of the zygote formation at extraoptimal temperatures would likely be caused by the correlating action of temperature upon the mechanism of the plasmic membrane.(2)

(1) HARTMANN (1929, p. 92) remarks that the gamete of *Dasycladus* may change the sexual affinity, but not the sexual constitution, when the filtrate of the other medium of gametes is acted upon.

(2) An observation of polyspermy is in this respect worthy of record. A female was visited by two males almost simultaneously. While they were reacting to the female for 4 minutes, one of them began to fuse. No sooner did the fused mass become spherical, than the second male was fused with it, and at 6 minutes after the association of these three gametes a larger zygote resulted. It is characterized by having 3 equal-sized orange drops arranged at an equal distance from one another. In the

From the account so far given, we can draw the conclusion that in *Synchytrium fulgens* the individual distinction between both sexes as assumed in *Ectocarpus* does not exist. Absolute length of the swarm period indicates simply the length of the male stage in each gamete but nothing of the degree of the sex. Copulation is assured between gametes whose swarm period differs widely in length, and its difficulty is enhanced as the difference is becoming slight. It is the relative length of the swarm period that is decisive of the copulation of the gametes. With this assertion the so-called relative sexuality in our fungus may be illustrated as follows:

Assume sister gametes, A, B, C, and D, to have different lengths of the swarm period (the male stage), for instance, 10, 15, 40, and 50 minutes respectively. Then the copulation of A or B with either C or D is assured, but between A and B or between C and D it is not certain. A becomes sedentary 5 minutes earlier than B, so that it may happen that B, while reacting to A, becomes sedentary. Similarly, D may react to C just becoming sedentary and is able to behave as a male for 10 minutes. It is not sure that the copulation is effected within the 10-minute duration of reaction, though it would be more probable than within the 5-minute duration in the combination of A and B.

If HARTMANN's explanation is accepted in this case, A and B would be designated as females, strong (♀♀) and weak (♀) respectively, while C and D as males, weak (♂) and strong (♂♂) respectively. Then the result obtained from the combination of these gametes may be represented in an accompanying table (Table VII).

	♀♀ A	♀ B	♂ C	♂♂ D
♀♀ A	—	—	++	++
♀ B	—	—	++	++
♂ C	++	++	—	+
♂♂ D	++	++	+	—

TABLE VII

Combinations of gametes with different lengths of the swarm period (+ +, copulation sure; +, probable).

germination experiments hitherto carried out the zygote of a similar form has often been found here and there among the ordinary zygotes. Now we convince ourselves that polyspermy is not rare. It might be presumed that the modification of the physical nature of the plasmic membrane attains in this case a critical stage for fusion almost simultaneously in three gametes. It is interesting to note that polyspermy is found in several isogamic algae (cf. OLTMANNS, 1923, p. 130; JOLLOS, 1926, p. 282).

It induces that the copulation is effected (C × D) or the sexual affinity may be presented (A × B) between gametes of the same sex. It is in exact agreement with HARTMANN's conclusion upon *Ectocarpus*. However, invalidity of this induction is apparent from inconstancy of the sex character in each gamete.

Now turning to *Dasycladus*, with which JOLLOS justified HARTMANN's hypothesis, we will find that the swimming and sedentary periods are not marked in the copulating gametes, copulation taking place while both gametes are swimming. This feature may obscure the period of the sex alteration, even though such might take place. However, one of JOLLOS' extensive experiments appears very suggestive of the sex alteration. On combining freshly derived gametes with old ones (30–32 hours old) he (p. 255) obtained a remarkable result; the old − gametes retained the sexual action, being able to form the zygote with new + gametes, but the combination of the old + and new − gametes does not exhibit sexuality, showing, according to him, the disappearance of the sexual character in the old + gametes. Bearing in mind the case in *Synchytrium fulgens*, this striking fact gives us an idea that the gamete may be first +, but on becoming old may be changed into −, and what is assumed the − individual is one, in which the turning point from + to − falls on an early period, while the + individual represents one, in which the turning point falls on a later period.(1) Further, he found the interesting fact that the sex or its degree is changed through the action of a filtrate of the medium, in which old gametes existed. It appears to suggest that the turning point of sex might be shifted in any way by the action of substances existing in the filtrate, as does the temperature in the case of *S. fulgens*.

An assumption of the sex alteration in the gamete may throw some light upon an occasional monoecism in both *Ectocarpus siliculosus* and *Dasycladus clavaeformis*, which are generally known as dioecious. It is a remarkable fact that *E. siliculosus* is reported sometimes as monoecious and sometimes as dioecious by different observers at different seasons and localities (cf. KNIEP, 1928, p. 153). JOLLOS (p. 289) mentions in *D. clavaeformis* a seasonal variation in the occurrence of monoecious individuals.

Assuming that the sex character is altered in each gamete, dioecism would be the result, if all sister gametes have strictly the same

(1) JOLLOS (p. 290) gives that the gametes are generally able to copulate still at 24 hours after liberation.

length of the male or + stage. However, it is possibly assumed that under internal or external conditions there may occur individual variations regarding the period of the sex alteration (from male to female or from + to −). Then there may occur a critical period, at which some of the sister gametes face others having the opposite sex, manifesting monoecism. JOLLOS (p. 292) notices that the monoecious copulation occurs later than usual. This delayed copulation appears to be explicable, if we assume that all gametes are + at earlier periods of life but at a certain later period some become − earlier than others, as ascertained in *S. fulgens*.

The principle of the sex differentiation underlying the relative sexuality has received a great deal of attention, and a similarity of the relative sexuality in the algae and the pluripolar sexuality in Hymenomycetes is often the subject of discussion (KNIEP, 1928; HARTMANN, 1929). However, from the consideration given in the foregoing it would appear desirable that the relative sexuality should be substantiated with further accurate observational facts before a theory is formulated upon it.

2. The Life-Cycle and the Mode of Development in *Synchytrium*

The genus *Synchytrium*, comprising comparatively a large number of species mostly parasitic on land plants, presents a certain variation in the life-history and in the mode of development. The characteristic points on this respect may be mentioned as follows:

1. Two life-cycles are distinguished. The one is represented by the summer form, in which the gametangium sorus is invariably produced, while the other involves the hibernating stage, being represented by the resting cell. Typically the life-history is completed on presenting both cycles, but there are exceptions, in which one of them is omitted.

2. The gametangium sorus in the summer cycle is in one case exogenous in origin, that is, it is produced outside the vegetative fungus body. In the other case the fungus body directly develops into the sorus, thus showing an endogenous origin.

3. The gametangium derived from the resting cell follows the same modes of formation. In some species the sorus is formed outside the resting cell, while in others a single gametangium is formed inside it.

Referring to those members of the genus that have been thoroughly investigated, the life-history of each species is presented in various combinations of different life-cycles and different modes of the gametangium formation. In this respect the members of the genus are classified in the following five types (Table VIII) :—

TABLE VIII

Type	Summer sorus	Winter sorus	Species
I	Exogenous	Endogenous (single gametangium)	*S. endobioticum* (SCHILB.) PERCL. *S. Succisae* DE BARY et WORON. *S. Stellariae* FUCKEL.
II	Endogenous	Endogenous (single gametangium)	*S. taraxaci* DE BARY et WORON.
III	Endogenous	Exogenous	*S. fulgens* SCHRÖT.
IV	Wanting	Exogenous	*S. aureum* SCHRÖT *S. mercurialis* (LIBERT.) FUCK. *S. globosum* SCHRÖT. *S. rubrocinctum* MAGN. *S. alpinum* THOMAS. *S. potentillae* (SCHRÖT.) LAGH.
V	Endogenous	Wanting	*S. Puerariae* MIY. (*S. minutum* [PAT.] GM.) *S. decipiens* FARL. (*S. aecidioides* [PK.] LAGH.)

Regarding the genetic connection between the summer and the winter cycles, an exact account has not yet been given. That the winter cycle originates from the zygote is demonstrated by CURTIS (1921) in *Synchytrium endobioticum*, but it is not clearly shown whether the winter cycle is exclusively succeeded by the summer cycle or whether it may also give rise to the next winter cycle. In *S. fulgens* an attempt has been made to elucidate this point. After following out

the life-history completely it has been ascertained that both the summer and the winter gametangia may produce the zygote and azygote, so that both cycles may originate from the resting cell. The connection between the two cycles may be represented in an accompanying figure (Fig. 19).

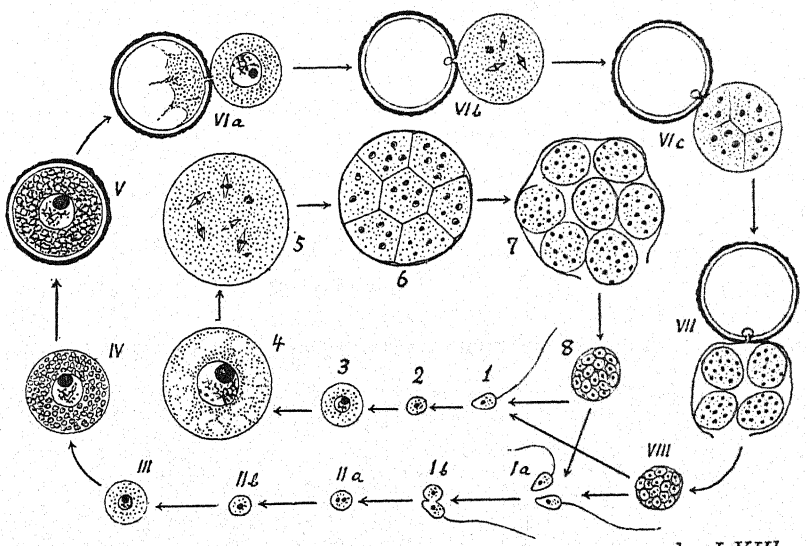


Fig. 19. Life-cycle of *Synchytrium fulgens* (*1–8*, summer cycle; *I–VIII*, winter cycle).
1, a parthenogenetic gamete; *2*, its encystment; *3*, *4*, growing stages in the host-cell; *5*, multiplication of nuclei; *6*, segmentation into gametangia; *7*, summer sorus; *8*, summer gametangium in germination. *Ia*, two gametes; *Ib*, their copulation; *IIa*, zygote; *IIb*, zygote with a fused nucleus; *III*, *IV*, growing stages in the host-cell; *V*, adult resting cell; *VIa*, its germination; *VIb*, stage corresponding to *5*; *VIc*, stage corresponding to *6*; *VII*, winter sorus; *VIII*, winter gametangium in germination.

Throughout an entire vegetative season, so far as observed in our climate, the summer cycle may involve more than 10 generations. Also, in the winter cycle at least two generations may be repeated. The over-wintering resting cell germinates in the spring. The resting cell formed during the spring remains upon the host plant till the summer. Afterwards the diseased portion begins to die, and towards the autumn it is rotten enough to set free the resting cells upon it into soil. At this period the seeds of the host produced in the summer time are about to germinate, and also the resting cells are able to germinate and cause the soil infection of the seedlings. New resting cells thus formed can mature before the vegetative season is closed.

3. The Sexual Feature in *Synchytrium*

The reproductive cell and organ of *Synchytrium*, which had for a long time been considered as being asexual and had been called the zoospore and sporangium, have been treated in the present paper as being sexual, using the term of the gamete and gametangium respectively. Yet, as the main phase of the life-history is represented by the asexual reproduction, the general application of the revision of terminology may be subject to discussion.

So far as investigated in *S. fulgens*, the zygote formation takes place under conditions which, judged from the behaviour of the reproductive cell, may be considered ordinary. Therefore, the sexual function should be taken as typical, the asexual reproduction being accessory.

Speaking generally, every reproductive cell expresses the sexual action leading to copulation, developing however asexually if unsuccessful of actual fusion. It gives a strong evidence for the gametic nature of all individuals of the reproductive cell.

A cytological study of this fungus reveals the fact that the summer sorus represents the haploid generation. It originates from a single reproductive cell which is, as stated above, able to give rise to the diploid generation on forming the zygote. As a matter of fact, the reproductive cell to be derived from the summer sorus is haploid and it develops again into the haploid sorus or is able to form the diploid zygote on copulation. The reproductive cell descended from the resting cell is in all respects quite similar to that derived from the haploid summer sorus. A cytological study of the germinating resting cell reveals the reduction division, distributing the haploid number of chromosomes among the reproductive cells derived from it.

On these grounds we look upon the asexual reproduction presented in *S. fulgens* as the parthenogenetic development of the gamete.

In the light of sexuality attention may be drawn to the various types of the life-history in the members of *Synchytrium*. In one type the life-history is completed through both the summer and the winter cycles. TOBLER's subgenus *Pleiochytrium* comprises the members of this type. From the study of *S. endobioticum* and *S. fulgens*, both belonging to *Pleiochytrium*, we believe that the resting cell in other members of this type is a sexual product and the gamete is capable of parthenogenetic development.

As representing the second type we mention *S. Puerariae* (*S. minutum*) and *S. decipiens* (*S. aecidioides*). The resting cell is still un-

known and their life-cycle is represented by the generation of the summer sorus. While in *S. decipiens* the manner of hibernation is as yet obscure, in *S. Puerariae* the summer sori deeply seated in the cortical tissue of the perennial stem of *Pueraria Thunbergiana* are able to over-winter safely and to cause the primary infection in the next year (KUSANO, 1908). Careful observations fail to find any indication of the copulating action in the swarm cells of this fungus. In the mode of formation the reproductive cell and organ of two fungi just mentioned appear to be homologous respectively to the gamete and gametangium of *S. fulgens* and consequently to be haplophase. However, on account of their lacking the sexual act one may assume that the reproductive cell concerned is diploid. According to my investigation (KUSANO, 1909), 5 chromosomes are constantly found in *S. Puerariae* throughout its life-cycle. This odd number seems to point to its being haploid and the fungus to be a haplont.(1) We come therefore to the view that in the nuclear feature the swarm cell of *S. Puerariae* is homologous with the gamete of *S. fulgens* and in fact is a gamete, whose sexual action is absolutely lost, becoming an obligatory parthenospore.

As the third type may be mentioned the members belonging to TOBLER's subgenus *Haplochytrium*. They are characterized by having only the winter cycle ; the fungus bodies invading the host in the spring produce the resting cell which, like that of *Pleiochytrium*, remains dormant till the next year. Unfortunately, we are as yet quite ignorant regarding sexuality, but from the account given of the preceding two types we infer that only the zygote is infectious, the gamete being strictly obligatory and incapable of parthenogenetic development.

Thus the gamete in *Synchytrium* may be assumed to exhibit its sexual function in varying degrees according to different species. In some it may be obligatory, while in others it becomes an obligatory parthenospore. Besides, there are many species, in which asexual and sexual functions are equally present.

On this assumption it may be admitted to state that the member of the genus, which possesses obligatory isogametes, if such truly exist, may be regarded as the original or most primitive form and that it has derived along the retrogressive line a form having the facultative gamete and further a form whose gamete has lost the sexual property.

(1) In *S. decipiens* 4 chromosomes are found during the nuclear divisions in the prosorus, while in *S. endobioticum*, according to CURTIS (1921) and *S. fulgens*, according to my investigation, the haploid number is 5.

One may advance a quite opposite view, viz. the most primitive member of *Synchytrium* is sexless. Along the progressive line of evolution there is derived a member whose zoospore acquires a character to perform sexuality, as may be exemplified by *S. fulgens*. As the sexuality here is most primitive as far as we know at present, *Synchytrium* may appear to exhibit an initiation of sexuality from asexuality. We must, however, recall to mind the current view that the Archimycetes, in which *Synchytrium* is included, is generally considered to have been derived from the Flagellata or the Myxomycetes, in both of which sexuality is developed. It is justifiable to consider that the origin of sexuality should be sought in organisms more primitive than *Synchytrium*.

From the consideration so far attempted it will be seen how important and interesting is the study of the subgenus *Haplochytrium* regarding the sexual problem. Further, it is emphasized that the study of *Synchytrium* along the line of sexuality would throw more light upon the phylogenetic relationship among the members than has hitherto been thrown.

IX. Summary

1. In *Synchytrium fulgens* the sexual reproduction takes place. The zoospore, as hitherto called, is in fact the planogamete. The zygote derived from two copulated gametes develops into the resting cell, while a single gamete is able to develop parthenogenetically into the summer gametangium.

2. The resting cell produces on germination the winter gametangium sorus. It is exogenous in origin, while the summer sorus is endogenous. Both forms of the sorus as well as the gametangium are similar to each other in structure and function.

3. The planogametes reveal no morphological difference from each other. But at the time of copulation one of the gamete is sedentary, becoming spherical, while the other is active, being pear-shaped. The sedentary one acts as a female and the active as a male.

4. Copulation may take place between the gametes derived from the same summer gametangium. The fungus is, therefore, monoecious.

5. Sexually considered, no individual difference is recognized in the gametes. Each gamete behaves as a male during the early and active period and as a female during the later and sedentary period.

Here the gamete exhibits consecutive monoecism in itself, so that the sex differentiation is expressed in terms of the life stages of each gamete.

6. Any external and internal condition favourable for the germination of the gametangium and for the activity of the gamete favours the zygote formation and *vice versa*.

7. In performing the sexual and asexual reproduction the gamete originated from the resting cell reveals nothing different from that derived from the summer sorus.

8. The resting cells stored air-dried may retain their viability for 7 years, and those kept in water may remain dormant for $3\,^1/_2$ years.

9. The viability of the gametangia from both summer and winter sori is retained more than 3 months, if kept at low temperatures being protected from dessication.

10. The resting cells can germinate without over-wintering or without being exposed to drought. They can also be brought to germination without a notable period of rest.

11. The zygote formation is controlled by temperature, 20°C. being optimal. Therefore, in the field the occurrence of the resting cells is chiefly influenced by the seasonal changes of temperature.

12. Among zygotes and azygotes entering the same host-cell competition for development prevails. When the host-cell is less vigorous, the zygotes survive for the resting cells; otherwise they are suppressed in development and the azygotes succeed in developing into the summer sori.

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Abstracts Nos. 1—89

(Referring to the principal papers on Botany and allied subjects which have appeared in Japan chiefly during July-December 1929)

1. Über die wildwachsenden gefülltblütigen Stöcke von *Gardenia jasminoides*, ELLIS. Toichi ASAI. (Japan. Jour. Bot. **4**, 1929, 335–344, 4 Textfig.)

2. Maïs et Mutation. (En japonais). Louis BLARINGHEM. (Bot. Mag. Tôkyô **43**, 1929, 355-367,8 figs. dans le texte).

La traduction japonaise d'une conférence donnée par l'auteur français concernant ses travaux bien connus sur la mutation du maïs provoquée par les traumatismes.

3. On the Physiological Difference between the Spring and Winter Types in Wheat and Barley. (Japanese with English résumé). Nakae ENOMOTO. (Jour. Imp. Agric. Exp. Sta. **1**, 1929, 107–138, 3 pls.).

The experiments were performed with 36 wheat and 105 barley varieties during 1924–26.

Sowing was made in spring at several different periods, and for each type the very first period of sowing was determined, whereby it is compelled to show the phenomenon of "Sitzenbleiben" (i.e. not heading out)—limit of "Sitzenbleiben." Various variants were observed by the author in this respect.

In the autumn sowing experiments the results of ordinary field culture, and those of greenhouse culture with or without night illumination were compared to each other. It was ascertained that both in wheat and barley high temperature accelerates the heading, and somewhat in higher grade in the former than in the latter. In respect to the accelerating effect of illumination wheat is in general less sensitive than barley, and there exist some wheat varieties, of which the heading is considerably detained by illumination. The following remarkable correlation, though not complete, was found: the higher the grade of the response, either to high temperature or the illumination, the higher the grade of spring growing habit (i.e. the greater the possibility of heading out by late spring sowing).

4. Mutation of the Endosperm Character in Rice Plant. (Japanese with English résumé). Nakae ENOMOTO. (Japan. Jour. Gen. **5**, 1929, 49–72).

A glutinous variety of rice, called "Aikokumoti" gives by selfing a few non-glutinous seeds which amount to $M \pm \sigma = 1.01 \pm 1.08\%$. Such mutant seeds are always heterozygous, and consequently they give in the next generation non-glutinous and glutinous offspring in 3:1 ratio. These non-glutinous offspring give in their turn the segregating and the constant non-glutinous families in the approximate ratio 2:1. In these segregating families, though the ratio of segregation is nearly 3:1, the recessive offspring (i.e. glutinous) are always somewhat less than theoretically expected (nearer to 24% than to 25%). This is due according to the author's

view to the gene mutation **g** (glutinous) to **G** (non-glutinous) in the gametes. In some families the recessive seeds are in excess than theoretically expected, and this is not explainable by the said mutation.

5. Über den Nährwert der Ammonium- und salpetersauren Salze als Stickstoffquelle bei der Wasserkultur von Reispflanzen. (Japanisch). Sadayoshi FUKAKI. (Proc. Crop Sc. Soc. Japan **5**, 1929, 47–51; auch Bult. Sc. Fak. Terk., Kjuŝu Imp. Univ. **3**, 1929, 243–262 m. deutsch. Zfg.)

Bei der Wasserkultur der Reispflanzen wurde es beobachtet, dass sie gut gedeihen und fruktifizieren können, wenn man bloss die Ammonium- oder die salpetersauren Salze als Stickstoffquelle benutzt, vorausgesetzt, dass die Reaktion der Nährlösung immer für die Kultur geeignet gehalten wird. Wenn Ammonium als Stickstoffquelle benutzt wird, geschieht das Wachstum der vegetativen Organe viel üppiger als wenn die salpetersauren Salze in Gebrauch genommen wurden. Es scheint es ganz umgekehrt zu sein bei dem Samenausreifungsvorgange.

6. Preliminary Report of the Serological Examination on *Brassica*. Eiji FUKUSHIMA and Yoshio MARUYAMA. (Proc. Imp. Acad. **5**, 1929, 473–476).

The serological examination of eight species of *Brassica* as well as *Raphanus sativus* according to the method of precipitin tests has shown that from the serological point of view they may be classified into four groups, viz. 1. *B. japonica, Rapa, pekinensis* and *chinensis*, 2. *B. Napellus*, 3. *B. juncea*, 4. *B. oleracea* (*Raphanus sativus*). This classification is in accordance with the results of recent genetico-cytological studies.

7. On the Rôle of the Factors C and R in the Production of the Flower Colours in *Pharbitis Nil*. Tokio HAGIWARA. (Bot. Mag. Tôkyô **43**, 1929, 643–656).

By means of a series of crossing experiments the author has ascertained the genetic composition of some strains of the Japanese morning glory as follows:

Cr.....white flower with coloured tube; stem green.
cR.....white flower with light yellow tube; stem coloured.
cr.....white flower with light yellow tube; stem green.

From above one can see that **C** is connected to some extent with the colour of flower-tube, and **R** with that of stem.

The author has detected further a factor **Ca**, without which even the plants provided with **C** and **R** produce white flower with yellow tube, green stem and white seeds, thus, for instance, **ca CR, ca Cr, ca cR** belong to such class of plants.

8. Über die Beeinflussung des Wachstums des Mesokotyls und der Koleoptile von *Avena*-Keimlingen durch das Licht. Hideo HAMADA. (Proc. Imp. Acad. **5**, 1929, 438–441, 5 Abb.).

Die hemmende Wirkung des Lichtes auf das Wachstum des Mesokotyls und der Koleoptile von *Avena sativa* -Keimlingen, sowohl bei verschiedener Stärke als bei verschiedener Wirkungsdauer des Lichtes wurde untersucht. Z. B. bei 30 Min. langer Belichtung mit 1262-MK. um 25° zeigten die im 51-stündigen Alter gereizten Keimlinge in der 120-stündigen Endlänge den minimalen Wert der Mesokotylentwickung, sodass die Mesokotylkurve V-formig verläuft—was der Verf.

Hemmungskurve nennt. Erst bei der 24-stündigen Belichtung konnte der Verf. praktisch mesokotylfreie Keimlingen bekommen.

9. A New Theory on the Construction of Polycyclic Steles. (Japanese). Bunzô HAYATA. (Bot. Mag. Tôkyô **43**, 1929, 340–355, 8 figs.)

The author starts with the theory hitherto put forth for the interpretation of polycyclic steles. According to this theory the steles, though they seem generally to possess a very complicated structure, are in brief in the case of dictyosteles composed of a number of meristeles arranged on several imaginary concentric cylinders. Consequently, when observed in the section of rhizome, the meristeles are considered to be disposed on certain concentric imaginary rings which correspond to the sections of the cylinders just mentioned. The author, by observing the so-called polycyclic stele extracted out bodily by himself by a special method from the rhizome of *Angiopteris evecta*, is inclined to make an interpretation quite contrary to that just mentioned. He has found that the same stele should be interpreted as composed from many imaginary conical wedges surrounded by meristeles. These wedges, each of which bears to each other the same mathematical relation as in phyllotaxis are inserted between each two ridges of an imaginary screw constructed, so to speak, by a strap of paper spirally revolving along the axis of the stele from the base to the top, either clockwise or counter-clockwise. The stele is according to the author by no means to be regarded as consisting of several concentric cylinders, as the word "polycyclic" indicates. The reason why the author here pictures to himself a wedge as an element composing the stele is that the rhizomes generally possess the form of a cone, of which the apical portion alone exists, the remaining being weathered out. If they are, on the other hand, columnar in shape, we should as a matter of course imagine a small cylinder in place of a wedge. Taking into consideration the more general cases, the author has formulated a new theory for the interpretation of the complicated structure of this kind of steles, proposing to call it the Wedge Theory. Now if the latter theory will be accepted, then we shall see that the view concerning the arrangement of meristeles observed in the section of a rhizome should be totally different from that of what has been put forth and hitherto supported. According to the author's theory, in the section of a rhizome there exists a certain number of the smaller sections of the imaginary wedges disposed on the fundamental spiral resembling an Archimedian or a logarithmic spiral, as the case may be, each of the wedges bearing to one another the same mathematical relation as in the phyllotaxis. The meristeles seen in the section of the rhizome are so arranged as to make several sets, each belonging to one of the smaller sections of the imaginary wedges, and the meristeles of each set are disposed on each smaller ring which answers to the smaller section of the wedges; these rings exactly correspond to leaf-gaps of the stele. In order to test the wedge theory the author has tried to interpret by means of his theory the figures of the so-called polycyclic steles which have hitherto appeared in the literature, such as those given by SHOVE, WEST, KUHN and BOWER on the steles of *Angiopteris*, *Danaea*, *Kaulfussia*, *Thysopteris* and *Saccoloma*, and he has found that not only may all such figures be easily elucidated by the new theory, but also a very clear mathematical significance may be given in respect to their otherwise quite disorderly arrangement of meristeles. Author.

10. On the Relation of Soil Moisture to the Development of the Rice Blast Disease. (Japanese). Takewo HEMMI. (Agric. & Hortic. **4**, 1929, 1143-1154, 1 pl.).

A certain number of inoculation experiments with the causal fungus of rice blast disease, *Piricularia Oryzae*, grown on the soil differing in its moisture was performed. From the results of such experiments, all of which were in perfect agreement to each other, the author draws the conclusion that the more humid the soil where the plant is growing, the lower the rate of infection, and the longer the duration of dry season the higher the latter also.

11. On the Possibility of Soil Infection of *Piricularia Oryzae* and Its Relation to Soil Moisture. (Japanese). Takewo HEMMI and Shigeru ENDO. (Agric. & Hortic. **4**, 1929, 773-784, 1 pl.)

As the result of several experiments the authors have attained the following conclusions. If *Piricularia Oryzae* enters the soil of the nursery of rice-plants it can penetrate into the roots of the latter and lead to their disease and final death. The possibility of infection is much influenced by the moisture retained in the soil. If water layer is present upon the soil of the nursery, the power of infection is very weak, which is due at least partly to the fact that the development of the fungus is much prevented on account of the want of oxygen.

12. Studies on *Fomes ulmarius* Causing the Heart-wood Rot of *Cryptomeria japonica*. (Japanese with English résumé). Takewo HEMMI, Shigekatsu HIRAYAMA and Tomowo NOJIMA. (Bot. Mag. Tôkyô **43**, 1929, 657-675, 9 text-figs.).

Fomes ulmarius FR., which the authors think to be identical with *F. geotropus* COOKE is widely distributed in Japan, and found parasitic on *Cryptomeria japonica* and many deciduous trees. The authors present the results of their investigations on this fungus parasitic on *Cryptomeria japonica*. It belongs to the group of lignin-dissolving fungi, and causes the brown pocket rot of the heartwood of that Conifer. These pockets or cavities are partially filled with yellowish brown powder, and occasionally also with white mycelium; masses of partially decayed wood are sometimes found together. Finally the pockets become empty.

Pure cultures are easily to be done. The fungus grows very vigorously at the temperature 24°-40°C, the optimum for the mycelial growth being ±36°.

The morphological characters as well as the taxonomy of the fungus are described.

13. Cytological Basis of the Sex Determination in *Cannabis sativa*, L. (With Japanese résumé). Kenji HIRATA. (Japan. Jour. Gen. **4**, 1929, 198-201, 2 pls.).

By studying the pollen mother-cells in male as well as male intersexual plants of two varieties of *Cannabis sativa*, viz. Tochigi and Karafuto, the author was able to discern in the metaphase of the heterotype division a geminus consisting of two unequal chromosomes. In the female intersexual plants he could observe a geminus which is larger than the others, and it was composed of two equal chromosomes. The author comes to the conclusion that in male and male intersexual plants there exists a XY-pair and in the female presumably a XX-pair, the smaller chromosome in the XY-pair being Y-chromosome.

14. The Melampsoraceae found in the Tundra-Regions in the Neighbourhood of Sisuka, Saghalien. (Japanese). Naohide HIRATSUKA. (Jour. Agric. and Dendrol. Soc. Sapporo **21**, 1929, 59–63).

10 species are given, each with a description.

15. Chrysomyxa of Japan. (Notes from the Melampsoraceae of Japan II). Naohide HIRATSUKA. (Bot. Mag. Tôkyô **43**, 1929, 466–478).

11 species of *Chrysomyxa* are enumerated, of which one, *C. alpina* is new and described.

16. Nuntia ad Floram Japonicam IV-V. Masaji HONDA. (Bot. Mag. Tôkyô **43**, 1926, 540–544, 656–657).

The following new species are described : *Rosa yatsugatakensis, Stellaria Franchetii* with some new varieties, *Carex Sekimotoi, C. pudica, Ranunculus yatsugatakensis* and *Erigeron Koidzumii.*

17. Studies on the Hepaticae of Japan. II. Yoshiwo HORIKAWA. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 395–429, 3 pls. and 15 text-figs.).

The following species are described in detail with illustrations. *Fimbriaria Yoshinagana* sp. nov., *Conocephalus conicus* (L.) NECKAR, *C. supradecompositus, Lunularia cruciata, Chomiocarpon quadratus, Blasia pusilla, Cavicularia densa, Calobryum rotundifolium, Ptilidium pulcherrimum, Lopholojeunea densiloba* sp. nov., *L. javanica, Nothotylas japonica* sp. nov., *Anthoceros gemmiferus.* The distinct sexual dimorphism in *Blasia* and *Cavicularia* is pointed out, where the female thalli are much larger than the male ones; this fact is represented both by the tables and the curves.

18. Studien über die Rostkrankheit japanischer Minze. (Japanisch). Suehiko IKATA. (Mitteil. aus d. landw. Versuchsst. Okayamaken **34**, 1929, 115 S., 15 Tafeln u. 7 Textabb.)

Die vorliegende ziemlich umfangreiche Arbeit, die durch eine Anzahl von sehr lehrreichen Abbildungen begleitet ist, betrifft einerseits den zytologischen und experimentellen Untersuchungen der auf japanische Minze (*Mentha arvensis* var. *piperascens*) schmarotzenden *Puccinia Menthae,* und andererseits der Bekämpfung dieses Schmarotzers. Bei demselben kann man die Aezidio-, die Uredo- und die Teleutosporengeneration unterscheiden, obgleich er streng autözisch ist. Die Spermogonien, die in der Aezidiumgeneration erzeugt werden, enthalten Spermatien, welche keimungsunfähig sind. Die Dauer der Keimungsfähigkeit beträgt nur 2–5 Wochen bei Aezidiosporen und viel länger bei Uredosporen, da sie bei den letzteren unter günstigen Aussenbedingungen mehr als 187 Tage betragen kann. Die Temperatur für die Keimung der Uredosporen liegt zwischen 25°-30°C, das Optimum ist ca. 18°C. Die Infektionsweise mittelst Aezidio- und Uredosporen ist ganz gleichartig. Die Infektion findet stets durch die Spaltöffnungen statt, die bei japanischer Minze nur auf die untere

Fläche der Blätter entwickelt sind, und niemals durch Epidermiszellen statt. Die keimenden Sporen produzieren zunächst die Appressorien, die aus ihrem unteren Teile bald feine Schläuche ("penetrating tube") austreiben. Die letzteren dringen durch die stomatäre Spalte in die Atemhöhle ein, wo die sog. substomatäre Bläschen entwickelt werden. Aus den letzteren sind die Infektionshyphen ausgetrieben, die in den Wirtgewebe eindringen und mittelst der daraus entwickelten Haustorienmutterzellen mit den Wirtzellen in inniger Berührung kommen. Die Spitze dieser Mutterzellen dringen danach durch die Zellhaut in das Zellinnern ein, um dort die Haustorien auszubilden. Bei den Infektionsexperimenten der gegen unserem Pilze ganz immunen *Mentha piperata* hat der Verf. die merkwürdige Tatsache beobachtet, dass die auf den Blättern fallenden Sporen dort gut keimen und der Pilz bis zum Stadium der Ausbildung der Haustorienmutterzellen fortschreiten kann, doch wegen des bald zu erfolgenden Todes der infizierten Wirtzellen kann der Pilz nicht weiter wachsen und bald zu Grunde gehen, offenbar wegen des Nahrungsmangels. Die Uredosporen können unter günstigen Aussenbedingungen überwintern und im nächsten Frühjahr die Infektion ausführen. Die Ursache der Teleutosporenbildung ist nach den experimentellen Resultaten des Verf. lediglich der niederen Temperatur zuzuschreiben.

19. Studien über die kreisfleckige Blattfallkrankheit von *Diospyros Kaki*. (Japanisch). Suehiko IKATA und Takesi HITOMI. (Mitteil. aus d. landw. Versuchssta. Okayamaken **33**, 1929, 36 S. m. 5 Tafeln).

Was man bisher überhaupt als Blattkrankheit von *Diospyros Kaki* benannt hatte, ist keineswegs einheitlich. Dabei gibt es zwei verschiedene Arten, die durch die Gestalt der Krankheitsflecke leicht voneinander unterschieden werden können, nämlich kreisförmige oder winkelige, von denen die erstere die gewöhnlichere ist. *Cercospora Kaki* E. et E. und *Mycosphaerella Nawae* HIURA et IKATA sind die Erreger der winkel- bzw. kreisfleckigen Krankheit. Der letztere Pilz kann während 58-100 Tage im latenten Zustand bleiben. Die Infektion geschieht durch die an der unteren Fläche des Blattes befindlichen Spaltöffnungen. Der Pilz, der interzellular verläuft, sendet feine Haustorien in die Wirtzellen aus, um schliesslich sie zum Tode zu führen. Die Überwinterung ist durch die Sklerotienbildung ermöglicht, und die daraus hervorgehenden Ascen werden in Juni sich ausreifen.

20. Ein Beispiel der Pfirsichnektarinenchimäre in Japan. S. IKENO und Y. NOGUCHI. (Jour. Coll. Agric., Imp. Univ. Tokyo **10**, 1929, 305-312, 1 Taf. und 2 Textabb.).

Die Produktion von Pfirsichen und Nektarinen an ein und demselben Baum und von Früchten, die teilweise aus diesen zwei verschiedenen Sorten zusammengesetzt sind—was die Verf.n hier Chimärenfrucht nennt—ist seit langem bekannt. Die Verf. erwähnen in der vorliegenden Mitteilung ein neues Beispiel davon, welches in Japan vorgefunden worden ist. Die anatomische Struktur des Pfirsiche, Nektarinen und Chimärenfrüchte sind geschildert und untereinander verglichen. Die Chimäre wird als eine sektoriale gedeutet. Einige Hypothesen, die die Produktion der Chimärenfrucht verständlich machen, sind erwähnt: danach dürften die Chimärenfrüchte keineswegs die Produkte des Pfropfens, sondern vielleicht dieselbe der vegetativen Spaltung der spontanen Hybriden, Pfirsich×Nektarine oder umgekehrten, in der F_1 - Generation sein.

21. Über Hydrobryum japonicum IMAMURA, eine neue Podostemonacee in Japan. Shun-ichiro IMAMURA. (Bot. Mag. Tôkyô **43**, 1929, 332-339, 1 Taf. u. 10 Textabb.).

Eine neue in Südjapan entdeckte Podostemonacee, *Hydrobryum japonicum* wird beschrieben. Wenn diese Pflanze dem *H. Griffithii* TUL. sehr ähnlich ist, unterscheidet sie dadurch in folgenden Hinsichten: 1. Die Narbe ist zugespitzt im Gegensatz zu *H. Griffithii* mit sehr variabeler Narbe; 2. die Anzahl der Blütenblätter beträgt 3-5, während sie bei *H. G.* 4-7 beträgt, und 3. sie ist durch einen sehr eigentümlichen, über Wasser hervorragenden zusammengesetzten Thallus ausgezeichtnet.

22. Karyological Studies of *Iris Kaempferi*, SIEB. et ZUCC. Sukeo INARIYAMA. (Japan. Jour. Bot. **4**, 1929, 405-426, 3 pls. and 4 text-figs.).

23. On the Formation of Chromosomes in Barley. (Japanese with English résumé). Choyo INOUYE. (Proc. Crop Sc. Soc. Japan **5**, 1929, 25-39, 2 pls.).

Sometime ago the author has observed in the meiotic division of pollen mother-cells in *Linum* the fact that in the course of chromosome formation from nuclear threads the whole structure becomes almost colourless, which he calls the achromatic stage. In the same division of the pollen mother-cells of barley he could observe the same phenomenon. It was observed that towards the end of the pachytene stage the spirem gradually loses its chromatin, and consequently the nuclear threads become almost achromatic, but when the two synaptic mates are separating the chromatin which has once disappeared gradually begins to reappear on the spirem. Hence the author concludes that there are two sets of chromatin, viz. those which appear early in the prophase (prochromatin) and those which appear afterwards, i.e. ordinary chromatin.

24. Symbolae ad Mycologiam Japonicam I-III. Tokutaro ITO. (Bot. Mag. Tôkyô **43**, 1929, 460-466, 515-524, 633-643).

An enumeration of Japanese species of *Aleurodiscus* (11 spp.), *Peniophora* (14 spp.), *Corticium* (10 spp.), *Glœocystidium* (2 spp.), and *Asterostroma* (1 sp.).

25. Cytological Studies on the Pollen-formation of the Hybrids between *Triticum* and *Aegilops*. Fuyuwo KAGAWA. (Japan. Jour. Bot. **4**, 1929, 345-361, 3 pls.)

26. On the Phylogeny of Some Cereals and Related Plants, as Considered from the Size and Shape of Chromosomes. Fuyuwo KAGAWA. (Japan. Jour. Bot. **4**, 1929, 363-383, 2 text-figs.).

27. A Study on the Phylogeny of Some Species in *Triticum* and *Aegilops*, based upon the Comparison of Chromosomes. Fuyuwo KAGAWA. (Journ. Coll. Agric., Imp. Univ. Tokyo, **10**, 173-228, 5 pls. and 10 text-figs.)

The comparisons of chromosomes in root-tips in four species of *Triticum* and two species of *Aegilops* were made and the phylogenetic relationships among different species in a genus were accordingly discussed. The comparisons of chromosomes were made on the basis of their length, number of constrictions and their relative positions in the chromosomes. The "projection method" devised by the writer was

employed in order to know the exact length of chromosomes which were located obliquely to the plane perpendicular to the microscopic axis and giving the foreshortened views. In root-tips of these materials fixed after the treatment by the dilute aqueous solution of chloral hydrate, the chromosomes appear considerably shorter and thicker, with less amount of foreshortening than those in ordinary roots. Thus the errors occurring in the determination of chromosome length can be much more reduced in treated roots than in those in ordinary ones.

In treated roots of *T. monococcum* (2x species, 2n=14), the length ratios among different chromosomes were ascertained to be about the same as those in ordinary material. Each chromosome shows one constriction at the relative position similar to that of the constriction located at the insertion point of the spindle fibre in the corresponding chromosomes in ordinary roots. Besides, there appears in certain chromosomes at definite positions another constriction which is latent and unobservable in ordinary roots. Consequently, the classification of chromosomes according to both the length and the state of constrictions can be made more easily in treated roots than in ordinary ones.

In treated roots of *T. polonicum* (4x species, 2n=28), *T. dicoccum* (4x species, 2n=28) and *T. vulgare* (6x species, 2n=42), one or two constrictions appear at the definite positions in each chromosome. In these 4x and 6x species, it has been found that the length ratios between chromosomes which are the longest and the shortest or nearly so in a set, are remarkably larger than the ratio between the longest and the shortest ones in a set of *T. monococcum*. The somatic chromosomes of *T. polonicum*, *T. dicoccum* and *T. vulgare* may be classified into at least 8, 10 and 9 types in the respective species, and in these types there are contained respectively 6, 7 and 8 types which are not possessed by *T. monococcum*.

It seems probable that certain chromosome types are contained in common in two or more species among *T. monococcum* and the 4x and 6x species. But the number of such chromosomes existing in common in *T. monococcum* and the 4x and 6x species is probably two in a cell of the 4x and 6x species, not showing double or triple of the number of the corresponding chromosomes in *T. monococcum*, which is 2x species. In *T. polonicum* there are only two shortest chromosomes, and no more in a somatic set. In the 4x and 6x species, the number of the homologous chromosomes is probably two in a cell for most of the chromosome types classified. It seems possible that one pair of chromosomes exists in each of these types.

Consequently, the chromosome sets of the 4x and 6x species may be regarded not to present the reduplication of that of *T. monococcum* as well as of any other basic 2x species. These 4x and 6x species were not phylogenetically formed by any possible method which involved the reduplication of a basic chromosome set, but they may have been derived from the crosses among ancestral forms having different chromosome contents, the special chromosome behaviors having taken place in the hybrid meiosis.

In *Aegilops cylindrica* (4x species, 2n=28) the length ratios of the chromosomes which are truly or almost the longest and the shortest in a set of treated roots, are remarkably larger than those in *A. speltoides* (2x species, 2n=14), showing that their chromosome sets are not in autopolyploid relation. Author.

Entry 27

28. On a Mutable Strain of *Celosia cristata* L. (Japanese with English résumé). Benso KANNA. (Bot. Mag. Tôkyô **43**, 1929, 407–413).

A striped strain of *Celosia cristata* described in this paper gives by selfing always ±5% self-colored magenta mutants. The latter are quite constant in later generations in marked contrast to what has been seen by TERASAWA (cf. Japan. Jour. Bot. **1**, p. (50), No. 118.—Ed.). Bud-variations are often observed: the ears which are either pure magenta or somewhat intermediate in color (in the latter each petal being the mosaic of yellow and magenta) are produced. Magenta ears which are accompanied by green stems give the offspring which are quite the same as those of the original striped strain. The intermediate-colored ears which are accompanied by colored stems give those which are different from those of the striped strain.

29. Illustrations of Japanese Fungi. Seiichi KAWAMURA. Tôkyô 1929.

This book embodies besides the preface (5 pp.), the contents (9 pp.), the general remarks on the classification of fungi (7 pp. with text-figures), and the index (6 pp.) the illustrations of Japanese fungi with the explanations. All illustrations, generally in natural size, were drawn by the author himself (except a few) in natural colours. The fungi, 242 in all, belong mostly to the Basidiomycetes, partly to the Ascomycetes, and some few to the Myxomycetes and the Fungi imperfecti. Though the explanations are written in Japanese, the coloured drawings may be of much interest even to foreign mycologists who do not understand the Japanese language.

30. Cytological Studies on *Iris*. Natsu KAZAO. (The Sc. Rpts. Tôhôku Imp. Univ. 4th Ser. (Biol.) **4**, 1929, 543–549, 3 text-figs.).

The haploid number of chromosomes in some species of *Iris* was found to be 12, 14, 16 and 18 respectively. In *I. Kaempferi, sibirica, laevigata* and *gracilipes* the meiotic division in the pollen mother-cells goes quite normally. In the prophase of heterotype division of those in *I. florentina* and *japonica*, however, a number of trivalents are met with, of which the daughter chromosomes are distributed unequally between the opposite poles, so that the pollen grains which are produced by them are irregular in size. It is to be added, that while *I. florentina* is found in Japan only in cultivated condition, *I. japonica* grows wild.

31. An Instance of Linkage of Sex-chromosomes and Autosomes in the Pollen Mother-cells of *Humulus japonicus*. (Japanese with English résumé). Hitoshi KIHARA. (Japan. Jour. Gen. **5**, 1929, 73–80).

The author gives a first example of the linkage between the sex-chromosomes and autosomes ever seen in plants. In the heterotype division of the male plants of *Humulus japonicus* there are usually 7 pairs of autosomes and a sex-chromosome complex composed of three parts. The author has found an unusual male example, where there are 6 bivalents and one pentapartite chromosome complex which is evidently composed of 3 sex-chromosomes $\mathbf{Y_1XX_2}$ united end to end and an autosome pair **ss**. Such chromosomes are united in the order $\mathbf{Y_1ssXY_2}$; the gametes are expected, viz. $6+\mathbf{s}+\mathbf{X}$, $6+\mathbf{s}+\mathbf{Y_1}+\mathbf{Y_2}$, $6+\mathbf{s}+\mathbf{Y_1}+\mathbf{X}$ and $6+\mathbf{s}+\mathbf{Y_2}$. The second division is equational.

32. Conjugation of Homologous Chromosomes in the Genus Hybrids *Triticum*×*Aegilops* and Species Hybrids of *Aegilops*. Hitoshi KIHARA. (Cytologia **1**, 1929, 1–15, 15 figs.).

The variation of the number of bivalent chromosomes in one and the same hybrid was often observed. In 6 kinds of F_1 hybrids between various species-hybrids of *Aegilops* the variation in the number of bivalents in pollen- and embryo-sac mother-cells was studied; to cite only one instance, in the pollen mother-cells of *Triticum Spelta*×*Aegilops truncialis* hybrids the number of bivalents (incl. a few trivalents) varies from 1–5, and in the reciprocal hybrids from 1–7, the rest being the univalents. In respect to the cause of this variation the author came almost to the same conclusion as BLEIER, i.e. that it may be chiefly due to external conditions, for instance, presumably the temperature.

33. On the Morphological Characters and Fertility in Some Species-hybrids of Wheat. (Japanese with English résumé). Hitoshi KIHARA, Shunjiro WAKAKUWA and Ichizo NISHIYAMA. (Japan. Jour. Gen. **5**, 1929, 81–87, 3 pls.).

Among various species-hybrids of wheat made by the authors the hybrid *T. aegilopoides*×*T. dicoccoides* and its reciprocal are very remarkable. They are less than 30 cm in height, and completely sterile. The culm does not tiller at all. The head consists of 4–5 spikelets and is very fragile.

34. Cytological Studies of the Genus *Linum*. (With Japanese résumé). Muneo KIKUCHI. (Japan. Jour. Gen. **4**, 1927, 202–212,2 pls.).

The karyological study of the pollen mother-cells in a number of *Linum* species has revealed the fact that they may be classified into 4 groups having 9, 15, 18 and 43 (?) haploid chromosomes respectively. *Linum usitatissimum* which belongs to the second of the above mentioned groups shows always n=15 and 2n=30, and was found never to vary in its chromosome number. The F_1 hybrid, *L. alpina* (n=18)×*L. perenne* (n=9) possesses the somatic chromosome number 27 (i.e. 18+9). In the heterotype anaphase of the pollen mother-cells 9 bivalents are found at each pole, while 9, presumably univalents are lagging on the spindle, and moving towards the poles at random. A few F_1 plants were observed; their somatic number was various, viz. 20, 28, 34.

35. Contributions ad Cognitionem Florae Asiae orientalis. Gen'iti KOIDZUMI. (Bot. Mag. Tôkyô **43**, 1929, 382–407).

An enumeration of Japanese Phanerogams which are new or newly named by the author. All new plants are described.

36. Über die harten Samen von *Astragalus sinicus* L. und *Robinia pseudo-acacia* L. Mantarô KONDÔ. (Ber. Ôhara Inst. landw. Forsch. **4**, 1929, 289–293).

Bei *Astragalus sinicus*, welches in Japan sowohl als Grünfutter als Gründünger viel benutzt wird, ist der Prozentsatz von harten Samen stark variabel. Er schwankt nach deren Farbe (z. B. schwarzgrün 44%, dunkelbraun 21,6%, gelblichbraun 19,6%, braun 3,6%), der Sorte, dem Reifungsgrade (z. B. fast gar kein hartes Korn bei ungenügend reifen Samen) usw. Der Verf. hat betreffend die Zeitdauer der Ein-

wirkung des Wassers auf harte Samen eine Serie von jahrelangen Untersuchungen ausgeführt. Danach können eine Anzahl von harten Samen jahrelang im Wasser liegen, ohne zu keimen, z. B. hat der Verf. einen beobachtet, der erst nach 16 Jahren im Wasser gekeimt hat.

Nebenbei sind die Resultate seiner Experimente über die harten Samen von *Robinia pseudoacacia* hinzugefügt.

37. Über die anatomische Struktur und die taxonomische Bedeutung der Spaltöffnungen bei einigen Farnkräutern I-II. (Japanisch m. deutsch. Zfg.). Takeo KONDO. (Bot. Mag. Tôkyô **43**, 1929, 544–555 m. 14 Abb., 595–605 m. 8 Abb.).

Bei den Farnkräutern können die Entwicklung, Anordnung und Gestalt der Spaltöffnungen nicht selten als charakteristische Merkmale für die Unterscheidung von Arten, Sektionen und Gattungen benutzt werden. Die folgenden sind die von dem Verf. angegebene Zusammenfassung.

Die aus *Blechnum nipponica*, *B. Hancockii* und *B. amabile* bestehende Gruppe lässt sich von derselben von *B. castaneum* und *B. Spicant* durch den Entwicklungsgang und die darauf folgende Anordungsweise der Spaltöffnungen unterscheiden. *Drymoglossum rotundifolium* unterscheidet sich von *D. microphyllum*, *D. carnosum* und *D. obovatum* durch die Gestalt der Spaltöffnungen, welche sich aus dem Unterschied im Entwicklungsgange ergibt. *Cyclophorus adnascens* ist durch eine eigentümliche Ausbildung der Spaltöffnungen charakterisiert, und somit lässt sich von den anderen japanischen Arten der Gattung unterscheiden. Bei *Aneimia*, *Lygodium* und *Schizaea* gibt es einige eigentümliche taxonomisch verwertbare Verhältnisse.

38. Über den Effekt der Anwendung der "Pulvermethode" für die Bestimmung des Stoffgehaltes im Pflanzenkörper. V. Vergleichende Bestimmungen des Kohlenhydrat- und Eiweissgehaltes. (Japanisch m. deutsch. Zfg.). Riichiro KÔKETSU, Hirosi KOSAKA, Tosio SATÔ und Teru HUDITA. (Bul. Sc. Fak. Terk., Kjuŝu Imp. Univ. **3**, 1929, 232–243).

Der Zucker-, Stärke-, und Eiweissgehalt in den Pflanzenkörpern unter verschiedenen Bedingungen wurden vergleichend bestimmt. Jeder Gehalt wurde sowohl in Prozenten des Frisch- und Trockengewichtes als im Gehalt pro Einheit Volumen Gewebepulvers angegeben. Es wurde dabei gefunden, dass die schliesslichen Resultate bei allen untersuchten Materialien viel übereinstimmender durch die Pulvermethode als durch die anderen Methoden kommen, woraus man sicher an der Zuverlässigkeit der Pulvermethode schliessen kann.

39. Die Beziehungen zwischen den verschiedenen physiologischen Erscheinungen der Pflanzen und den an verschiedenen Vegetationsorganen in Erscheinung tretenden Farbstoffen. I. Mitteilung. Über die Beziehungen zwischen der Anthozyanbildung und dem Wachstum von *Abutilon Avicennae*. Hirosi KOSAKA. (Jour. Dpt. Agric., Kyushu Imp. Univ. **2**, 1929, 207–240).

Bei beiden Sorten von *Abutilon Avicennae*, "Akaguki" und "Awoguki" färben sich die Stengeln und Blattstielen rötlich-violett bald nach der Keimung, was auf einen Anthozyanfarbstoff zuzuschreiben ist. Die Intensität der Farbstoffbildung ist der Wachstumsgeschwindigkeit umgekehrt und der Anhäufungsmenge der Assimilate in den Zellen direkt proportional. Durch Besonnung wird die Farbstoffbildung beför-

dertz ; die Ursache dafür liegt erstens in der reichlichen Produktion der Assimilate (d.h. indirekte Wirkung) und zweitens in der direkten Wirkung des Sonnenlichtes.

40. Resistance of *Oenothera* to the Attack of *Synchytrium fulgens*. Shunsuke KUSANO. (Jour. Coll. Agric., Imp. Univ. Tokyo **10**, 1929, 313–327, 1 pl. and 2 figs.).

In nature the author could observe the fact that *Oenothera Lamarckiana* and *O. biennis* are affected by *Synchytrium fulgens*, but that *O. odorata* and *O. sinuata* growing together with the two above species remain free from the attack.

The experiment has proved that though *O. odorata* is found unaffected in nature, it may be seriously affected by the fungus, when cultivated under moist experimental condition. It was concluded that the thick tough wall of epidermal cells hinders in nature the penetration of the fungus, but that under moist condition the wall becoming hygrophyllous is so weak that it is no more able to resist its penetration.

In *O. sinuata*. if after inoculation the epidermal cells are examined, we will see within them the fungus bodies, indicating thus that the fungi can penetrate them. But while in cells of susceptible species, as *O. Lamarckiana* and *O.biennis* the fungus bodies as well as the nuclei and cytoplasma of the host cells are found growing conspicuously, in those of *O. sinuata* both are found in shrivelled condition, and we will see that finally both go to degeneration. It may therefore be concluded that in *O. sinuata* the inoculated host cells undergo at first the degeneration, and this leads to the death of the fungus on account of mal-nutrition. The immunity of plants is thus secured.

Further experiments have proved that even in *O. Lamarckiana* and *O. biennis* neither root-cells nor subepidermal cells are to be inoculated by *Synchytrium fulgens*.

41. On the Diagnosis of Certain Plant Infectious Diseases by Means of Serological Reactions. (Japanese with English résumé). Takashi MATSUMOTO. (Jour. Soc. Trop. Agric., Taihoku Imp. Univ., Taiwan **1**, 1929, 14–22).

To see, whether the diagnosis of certain plant infectious diseases could be made by means of serological reactions, as now much prevalent in human pathology, the author has inoculated rabbits with the suspensions of certain bacteria and got immune sera. The positive results were obtained only when antisera were added to the homologous antigens. The author therefore concludes that the serological method may be applied to identify or diagnose certain plant infectious diseases.

42. Studies on Some Phytopathogenic Bacteria with Special Reference to Agglutination and Complement Fixation. (With Japanese résumé). Takashi MATSUMOTO. (Jour. Soc. Trop. Agric. Taikoku Imp. Univ., Taiwan **1**, 1929, 155–171).

By means of various serological processes, as agglutination, agglutin absorption, and complement fixation the identification of some phytopathogenic bacteria from different sources was tried. To cite only some examples, No. 219 from tomato-wilt was found to be identical with No. 229 which is extracted from tobacco-wilt; again, the soft rot of *Brassica pekinensis* and other Cruciferae is identical with Nos. 212 and 197 extracted from *Cucumis Melo* and *Zinnia elegans* respectively.

The results of the agglutination and the complement fixation agree to each other essentially. In general the reaction of the latter is more active than that of the former, though less specific in differential ability.

43. Bibliographical Monograph on Plant Genetics (Genic Analysis) 1900-1925. Hajima MATSUURA. Tokyo 1929, 499 pp.

The book is divided into two parts. Part I, entitled Genic Analysis of Plants, embodies the results of crossing experiments of plants by various authors of the whole world from 1900 to 1925. The plants are arranged in the alphabetical order of their Latin names. Part II is devoted to the enumeration of literature consulted, 1341 in all, very often with some comments; it is arranged in the first place according to the year of publication, and in each year according to the alphabetical order of the authors' names. The index of subjects and that of species terminate the book.

44. Studien über die Zahlenverhältnisse der Chromosomen bei der Gattung *Viola*. Y. MIYAJI. (Cytologia **1**, 1929, 28–58, 64 figs.).

Der Verf. hat die Chromosomenzahl von 54 Arten, 6 Varietäten und 1 Form bei der Gattung *Viola* festgestellt. Hauptsächlich auf Grund solcher Angaben kann man schliessen, dass die haploide Chromosomenzahl der Gattung *Viola*, welche sehr mannifaltig ist, 6, 10, 12, 13, 17, 18, 20, 24, 27, 30, 36 oder 48 beträgt. Die Sektionen Dischidium, Chamaemelanium und der grösste Teil von Nominium gehören zu der 6 eren Reihe, wenn bei der letzteren keine 6-chromosomige Art vorhanden ist, und grösstenteils die Zahlen der 12 eren Reihe führen. Die artenreiche Gruppe Plagiostigma ist die einzige unter den 12-reihigen Formen, welche polyploide Beziehungen aufweisen, nämlich n=24 oder 36. Die Gruppe Langsdorffi ist durch die höchste Chromosomenzahl unter *Viola* ausgezeichnet, nämlich 48. Die Gruppe Diffusae weist n=13 auf, sodass sie unter der Gattung isoliert dasteht. Die anderen vier Gruppen Curvopedunculatae, Mirabilis, Silvestres und Canınae führen je 10 Chromosomen.

Ein Versuch über die Phylogenie der *Viola*arten mit Rücksicht auf die Chromosomenzahl wird gemacht, und das ganze Verhältnis schematisch dargestellt.

45. On the Inheritance of "Matusima"-Variegation in the Japanese Morning Glory. (Japanese). Bungo MIYAZAWA. (Japan. Jour. Gen. **4**, 1929, 167-184, 4 figs.).

The so-called "Matusima"-variegation in the Japanese morning glory is charactererized by having green and yellow patches of more or less great extension, which are as a rule sharply delimited from each other. Branches with purely green or yellow leaves may sometimes appear.

The selfing of the plants with Matusima-variegation, which are never the homozygotes, gives rise to green, Matusima-variegated and yellow. Greens extracted from Matusima-variegated plants partly throw green offspring exclusively (homozygotic), partly green+Matusima-variegated+yellow. The selfing of yellows shows that they are not only never homozygotic, but also genotypically quite identical with Matusima-variegated, because they segregate out three kinds of offspring, just as in the case of the latter. Presumably two genes are necessary to produce green colour; yellow is the result of the loss or inactivation of one of them. The latter will be frequently reactivated, and the Matusima-variegation may be considered as yellow, where some green patches were produced by this activation. The ratio of segregation of extracted heterozygotic green is for instance green: Matusima: yellow=

82.6:14.2:3.2, so that the number of greens is too large, if we will consider green: Matusima+yellow=3:1; this is due to the fact that yellows will often transform themselves into greens.

The crossing of Matusima-variegated with homozygotic yellow, and the examination of F_1 and F_2 hybrids has shown that the former is dominant to the latter.

46. Interspecic Hybridization in *Brassica*. I. The Cytology of F_1 Hybrids of *B. Napella* and Various Other Species with 10 Chromosomes. T. MORINAGA. (Cytologia **4**, 1929, 16–26, 4 pls.)

The cytological observation was done on the pollen mother-cells of some hybrids between *Brassica Napella* with 19 haploid and some other species with 10 haploid chromosomes (*B. pekinensis, Rapa, chinensis* var. *parachinensis, japonica*). In these hybrids 10 bivalents and 9 univalents are clearly distinguishable. The former take the regular position on the equatorial plane, while the latter are scattered on the achromatic figure. In the heterotypic anaphase the bivalents first disjoin and the daughter chromosomes approach both poles. The univalents undergo the splitting, and the daughter halves go rarely to the opposite poles, lagging chromosomes being excluded from the nucleus. In the homotypic anaphase most chromosomes divide and pass regularly to the poles, while few which are regarded as the halves of univalents divided in the previous division are left undivided in the early anaphase.

47. Chromosome Number of Cultivated Plants II. T. MORINAGA, E. FUKUSHIMA, T. KANO, Y. MARUYAMA and Y. YAMASAKI. (Bot. Mag. Tôkyô **43**, 1929, 589–594, 23 figs.)

Either the haploid or the diploid number of chromosomes in 25 spp. of cultivated plants was counted, and illustrated in this paper.

48. Notulæ ab Plantas Japoniæ & Koreæ XXXVII. Takenoshin NAKAI. (Bot. Mag. Tôkyô **43**, 1929, 439–459).

New species and varieties of Korea and Japan described in this paper are as follows:

Aconitum jaluense KOM. var. *glabrescens* NAKAI
Aconitum kiusianum NAKAI
Aconitum yesoense NAKAI var. *Majimai* NAKAI
Aristolochia Kaempferi WILLD. var. *pallescens* NAKAI
Berberis amurensis RUPR. var. *brevifolia* NAKAI
Bobua Uiæ NAKAI
Elæagnus crispa THUNB. var. *subcoriacea* NAKAI & MASAMUNE
Elæagnus Takeshitai MAKINO var. *longifolia* NAKAI
Exocarpus boninensis NAKAI
Fraxinns chiisanensis NAKAI
Lespedeza sendaica NAKAI
Persicaria vernalis NAKAI
Premna luxurians NAKAI
Quercus serrata THUNB. var. *pseudo-variabilis* NAKAI
Rhododendron iyoense NAKAI

Scutellaria iyoense NAKAI
Sideroxylon boninense NAKAI
Skimmia repens NAKAI var. *leucocarpa* NAKAI
Spiraea nipponica MAX. var. *oblanceolata* NAKAI
Stellaria yesoalpina NAKAI.

The following are new additions to the flora of Japan, Saghaline, Japan proper, Liukiu or Formosa.

Acalypha australis L. var. *glareosa* NAKAI, comb. nov. (syn. *Acalypha pauciflora* var. *glareose* RUPRECHT) NAKAI —— Kiusiu
Euonymus lutchuensis ITO —— Kiusiu
Eurya acuminata DC. —— Kiusiu and Formosa
Eurya acuminata var. *multiflora* BL. —— Liukiu
Pinguicula villosa L. —— Saghaline.

Beside the above, the critical studies of all Japanese species of *Ammodenia*, *Minuartia*, and the varieties of *Dianthus superbus* were made. The species and varieties enumerated by the author are as follows:

Ammodenia oblongifolia RYDBERG var. *maxima* NAKAI, comb. nov., *Dianthus superbns* L. var. *latifolius* NAKAI, var. nov.
Dianthus superbus L. var. *longicalycina* WILLIAMS
Dianthus superbus L. var. *speciosus* REICHENBACH
Minuartia arctica ASCHERS & GRAEBNER var. *minor* (HOOKER) NAKAI comb. nov.
Minuartia imbricata (C. A. MEYER) var. *koreana* NAKAI, comb. nov.
Minuartia Joi (MAKINO) NAKAI, comb. nov.
Minuartia laricina (L.) NAKAI, comb. nov.
Minuartia subfalcata (REGEL) NAKAI, comb. nov.
Minuartia verna HIERN. var. *alpestris* (FENZL) NAKAI, comb. nov.
Minuartia verna HIERN. var. *leptophylla* (REICHENBACH) NAKAI, comb. nov.
Minuaotia verna HIERN. var. *pulchella* (BUNGE) NAKAI, comb. nov. Author.

49. The Genetics and Cytology of Certain Cereals. 1. Morphological and Cytological Studies on Triploid, Pentaploid and Hexaploid *Avena*-Hybrids. Ichizo NISHIYAMA. (Japan. Jour. Gen. **5**, 1929, 1–48, 1 pl. and 84 text-figs.)

Triploid, pentaploid and hexaploid hybrids of *Avena* species are made artificially. The two former are highly sterile, while the last ones are fertile. In respect to morphological characters some are intermediate between the two parents, while some are dominant to the corresponding ones.

In the first division metaphase of PMC in the triploid hybrid, *A. barbata*× *A. strigosa*, 7 bivalents (incl. trivalents) form a normal equatorial plane, and the daughter chromosomes pass regularly to both poles. Univalents divide equationally, and the two halves go to different poles, though frequently they are not separated and travel together towards one pole; at telophase they often disappear. In the metaphase of the second division monad and dyad chromosomes are occasionally found outside the equator. The latter are longitudinally divided, and the halves go to opposite poles, while the former which are undivided go at random to either pole. Pollen tetrads are mostly 4-celled, but many pollen grains are almost empty or scanty in content.

The behaviour of chromosomes in the pentaploid hybrids is similar to that of triploid ones, though there are some differences.

In PMC of the hexaploid hybrids 21 normal bivalents are found in the first division metaphase, though sometimes 1–4 univalents, etc. are seen besides.

The author finally compares the results of his studies on cytological relationships with the genealogical tree of THELLUNG, and discusses the agreement of both.

50. A New Disease of Elm, Caused by *Gnomonia Oharana* sp. n. Yosikazu NISIKADO and Hiroyoshi MATSUMOTO. (Ber. Ôhara Inst. landw. Forsch. **4**, 1929, 279–287, 3 pls.)

The final paper of the authors' preliminary publication written in Japanese. (Cf. Japan. Jour. Bot. **4**, 1929, (96), No. 309).

51. Zur Kenntnis der Befruchtung und Kornbildung bei den Reispflanzen. Yakichi NOGUCHI. (Japan. Jour. Bot. **4**, 1929, 385–403, 34 Textabb.)

52. Studien über die Entwicklung der Infloreszenzen und der Blüten der Getreidepflanzen. Yakichi NOGUCHI. (Jour. Coll. Agric., Imp. Univ. Tokyo **10**, 1929, 247–303, 46 Abb. in Text.)

Die Entwicklunggeschichte der Infloreszenzen und der Blüten bei sechs Sommergetreiden (Reis, Mais, Sorghumhirse, Rispenhirse, Kolbenhirse und Getreidefennich) und vier Wintergetreiden (Gerste, Weizen, Roggen und Hafer) wurden studiert. Bei jedem Getreide befindet sich die Blütenständeanlage schon im Samen fertig und eine enge positive Korrelation besteht zwischen der Grösse des Embryos und der Blütenständeanlage. Betreffend den Anwachs der Blütenständeanlage bei den Getreidearten, der je 10 Tage nach dem Säen gemessen worden ist, gibt es im allgemeinen zwei Typen, d. h. Sommer- und Wintergetreidetyp. Bei dem ersteren findet meistens kein Anwachsen der Anlage während etwas 50 Tage (80 Tage bei Reis) nach dem Säen statt, und ihre gewaltige Vergrösserung, welche erst dann plötzlich eintritt, wird weiter bis zur Zeit der Entfaltung fortgesetzt. Bei dem letzteren wächst die Anlage dagegen während mehr als 120 Tagen sehr langsam und fängt dann plötzlich gewaltig sich zu vergrössern an. Die morphologische Veränderung der Blütenstände folgt im allgemeinen ihrer heftigen Vermehrung der Masse bei Sommergetreide, dagegen wird das erste Zeichen ihrer Differenzierung bei Wintergetreide schon einige Tage nach dem Säen sichtbar. Die auf Infloreszenz sich wachsende Blütenanlage aller Arten entwickelt sich in etwas ähnlicher Weise. Bei der Sommergetreide wird das Wachstum imnerhalb ganz kurzer Zeit beendet, aber bei Wintergetreide weit länger fortgesetzt. Keine nennenswerte Korrelation zwischen der Masse des vegetativen Wachstums und der Sexualorganentwicklung wird bei der Getreidepflanzen gefunden.

Der Verf. studiert noch den Anwachs der Rispenanlage von Reis unter verschiedenen Aussenbedingungen sowie den Geschlechtswechsel beim Mais. Verf.

53. Studies on Two Different Species of *Pestalozzia* Parasitic on the Leaves of *Diospyros Kaki* L. (Japanese). Tomowo NOJIMA. (Bull. Kagosima Imp. Coll. Agric. and Forest. **7**, 1929, 34 pp., 1 pl., 5 text-figs.).

Two kinds of diseases on *Diospyros Kaki* are announced, which lead to the premature falling of leaves in autumn. They are caused by *Pestalozzia Diospyri*

SYD. and *P. Theae* SAWADA respectively. The two kinds are distinguished by the shape and size of diseased spots, the shape of spores, the behaviour on nutritive media, etc. In the culture on dry apricot decoction solution what seems to correspond to pseudopycnidia is formed which the author regards as the young spore-layer concerned in its development. The temperature for the growth of both fungi lies between 10°–40°C, the optimum being 16°–32° for *P. Diospyri* and 24°–32° for *P. Theae*. The spores of *P. Diospyri* may live safely during 30 min. under 45°, but die after 20 min. under 55°.

54. Studien über die Erkennung der Drogen auf Grund des Aschenbildes. (I. Mitteil.). Aschenbilder der Drogenblätter im Pharmacopaea japonica IV. (Japanisch m. deutsch. Zfg.). Kametarô OHARA und Yosio KONDÔ. (Jour. Pharmac. Soc. Japan **49**, 1929, 1036–1043 u. 164–166, 1 Taf. u. 4 Textabb.).

Mittels der MOLISCHschen Aschenbildermethode werden eine Anzahl von Drogenblätter untersucht, z. B. folia Digitalis, Menthae, Salviae usw. Die Unterscheidungsmerkmale sind 1. die Krystalle, Drüsen, Krystallsand usw., 2. verkieselte Haare und 3. Aschenbilder der Epidermiszellen. Ein Schlüssel zur Bestimmung der 12 Arten Drogenblätter mittels dieser Methode wird angegeben.

55. On the Systematic Importance of the Spodograms of the Leaves of the Bambusaceae (VII). (Japanese). Kiichi OHKI. (Bot. Mag. Tôkyô **43**, 1929, 479–489, with figs.).

Continuation of his former publications. In this paper some species of *Pseudosasa* (*japonica, spiculosa, Owatarii*) are treated of.

56. Über eine tetraploide Gartenrasse von *Psilotum nudum*, PALISOT DE BEAUVOIS (=*P. triquetrum*, SW.) und die tripolige Kernteilung in ihren Sporenmutterzellen. Sakuichi OKABE. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 373–379, 1 Taf. u. 3 Textabb.).

Bei den Sporenmutterzellen von *Psilotum nudum* konnte der Verf. bei einer Rasse in der ersten meiotischen Teilung 52 Gemini nachweisen, wobei die Teilung ganz regelmässig vor sich geht. Bei einer anderen, welche 104 Gemini besitzt und daher als tetraploid anzusehen ist, beobachtet man in 80% Fällen die dreipolige Kernspindel, wobei die Gemini in drei Teile sich anordnen. Dabei werden nach der homöotypen Teilung viele Hexaden gebildet. Die reifen Sporen dieser tetraploiden Rasse sind ganz gesund.

57. Rhizoidentwicklung im Embryo von *Cystophyllum*. Sakuichi OKABE. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 591–595, 3 Textfig.).

Bei der Keimentwicklung von *Cystophyllum sisymbrioides* wird die Oospore zuerst in zwei fast gleichen Zellen quergeteilt, dann wird mittels einer Querwand eine linsenförmige Rhizoidzelle an einem Ende des Embryos gebildet. In dieser Zelle beobachtet man fünf sukzessive Zellteilungen, von denen bis zur vierten die Wände immer auf einer der vorhergehenden Wande fast senkrecht stehen. Die zentralen vier Zellen sind dabei viel schmäler als die anderen. Bei der fünften Teilung wird jede der letzteren zweistöckig quergeteilt. Nach dieser Teilung verlängern sich mit

Ausnahme der vier oberen Zellen in zweistöckigem Zustand alle 28 Zellen, um die Rhizoiden auszubilden. Vier obere Zellen erfahren noch eine Teilung, welche der Längsachse des Embryos parallel verläuft, und jede Zelle wächst zu einem Rhizoid. Etwas zwei Wochen nach der Oogonienentleerung verlängern sich sogar die untersten, an die inneren Rhizoiden angrenzenden Leibeszellen, um allmählich zu den Rhizoiden auszuwachsen.

Der Verf. studierte weiter die Keimesentwicklung von *C. Turnei* und beobachtete das gleichartige Verhalten.

58. Meiosis im Oogonium von *Sargassum Horneri* (TURN.) AG. Sakuichi OKABE. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 661–669, 3 Tafeln u. 2 Textfig.).

Die Reduktionsteilung im Oogonium von *Sargassum Horneri* wurde untersucht. Danach hat der Verf. 32 Gemini gezählt, was zur Angabe von KUNIEDA (s.z.B. Japan. Jour. Bot. **3**, 1927, (90), Nr. 267.-Red.) entgegengesetzt ist, wonach bei der Oogenese, der Spermatogenese und der Keimentwicklung die haploide und die diploide Chromosomenzahl dieser Art 16 bzw. 32 betragen sollen. Weiter konnte der Verf. in gewissen Entwicklungsstadien das gewöhnlich durch die Strahlung umgebene Centrosom nachweisen, welches schliesslich sich zu zwei teilt, obgleich KUNIEDA bei seinen Untersuchungen kein solches finden konnte.

59. Study of *Euryale ferox* SALISB. IV. On the Rate of Growth of the Lamina. Yônosuke OKADA. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 361–368, 2 pls.).

The leaves of *Euryale ferox* in some localities in Japan (for instance, Zyûnityôgata and Takaoka) attain not rarely the gigantic size not inferior to those of *Victoria regia*. The maximum growth takes place during the period extending from the latter half of August towards the beginning of September, when the temperature of air and water is very favourable for their growth. The author has measured the diameter and area of the leaf blade during such season, and found that the largest value found for the growth of the diameter attains 25 cm per day, i.e. 1 cm per hour. The ratio mass/area was also measured, and it was found that the mass increment declines considerably with the age.

The quantity of dry matter in percent of the fresh weight is almost constant for any sample. Its percentage is very low, as it is the general rule in aqueous plants.

60. Embryologie der Liliaceae, mit besonderer Rücksicht auf die Endospermbildung. I. Melanthioideae und Aletroideae. Tomowo ONO. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 381–393, 54 figs.).

Der Verf. hat die Embryosackentwicklung und die Endospermbildung einer Anzahl von Liliaceen (8 Melanthioideen und 1 Aletroidee) studiert. Die Embryosackentwicklung ist normal bei allen von ihm beobachteten Arten. Die Endospermbildung gehört teils zum helobialen (*Tofieldia japonica, Narthecium asiaticum, Metanarthecium luteo-viride, Veratrum Maackii, V. album* var. *lobelianum*), teils zum nukleären Typus (*Tricyrtis hirta, T. latifolia, Colchicum autumnale*).

61. The Variability of the Development of the Mechanical Tissue or Stereome in Leaves of Rice, and its Correlation in the Drought Resistance. (Japanese with English résumé). Jiro ONODERA. (Jour. Imp. Agric. Exp. Sta. **1**, 1929, 163–174, 2 figs.).

In the leaf-blade of rice-plants, which possesses the vascular bundles of various size, the stereome may be developed on its upper and lower side, though in the small bundles the upper is only feebly developed or not at all. In the varieties of lowland rice, the lower stereome is always very well developed, while in those of upland rice its development is much feebler, so that in this respect low- and upland rice could be clearly distinguished from one another.

62. Systematic Importance of Spodograms of Leaves in the Urticales II. (Japanese). Yosisuke SATAKE. (Bot. Mag. Tôkyô **43**, 1929, 413–421, 2 figs.).

Continuation of his former studies. The Moraceae are studied; they include *Artocarpus* (2 spp.), *Vanieria* (2 spp.), *Humulus* (2 spp.), *Cannabis*, *Fatoua*, *Morus*, *Malaisia* (each 1 sp.), *Broussonetia* (3 spp.), *Ficus* (2 spp.).

63. Physiologische Studien über den Winter- und Frühlingtyp von Gerste. I. Über den Unterschied der Keimung und Saugkraft. (Japanisch). Kenkiti SATÔ. (Proc. Crop Sc. Soc. Japan **5**, 1929, 42–46, 1 Kurve).

Die Keimung des Winter-, Frühling- und Zwischentyp der Gerste unter niederer (10°C) und höherer (20°C) Temperatur wurde untersucht. Bei höherer Temperatur geht die Keimung schneller vor sich als bei niederer, und dabei ist die für diesen Vorgang nötige mittlere Tageszahl bei drei Sorten fast gleich. Bei niederer Temperatur findet die Keimung des Winter- viel früherer statt als bei dem Frühlingtyp; der Zwischentyp stimmt in dieser Hinsicht im allgemeinen mit dem ersteren ein. Um zu untersuchen, ob die frühere Keimung der Wintergerste der grösseren Geschwindigkeit ihrer Wassersaugung zuzuschreiben ist, hat der Verf. in dieser Hinsicht einige Experimente ausgeführt, wonach entgegen seiner Erwartung diese Geschwindigkeit bei der Wintergerste kleiner als bei der Frühlinggerste vorgefunden wurde. Auch der Verf. hat ihre Keimung in der Zuckerlösung verschiedener Konzentrationen untersucht, um die Saugkräfte verschiedener Typen zu vergleichen, wonach es ergab sich, dass die Wintergerste auch dabei viel schneller keimt, dass ihre Keimungsprozent viel grösser ist als bei der Frühlingsgerste, und weiter dass der Zwischentyp in dieser Hinsicht mittelmässig steht.

64. Über die Wirkung der Elektrolyten auf der Sauerstoffverbrauch von *Chlorella ellipsoidea*. Mannen SHIBATA. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol) **4**, 1929, 431–471, 21 Abb.).

Die durch 0,4 ccm (frische Volumen) *Chlorella ellipsoidea* in der Nährlösung verbrauchte O-Menge beträgt während 90 Min. ca. 160–200 ccm, wenn in doppeltdestilliertem Wasser viel mehr O-Menge aufgebraucht wird. Konzentrierte Alkalikationen wirken auf dem O-Verbrauch schädlich, während verdünnte Alkalikationen beschleunigend wirken. Die Reihenfolge der beschleunigenden Wirkung ist wie folgt: K<Na<Li<Rb, während bei der Erdalkalikationen sie wie folgt ist: Sr<Mg<Ba <Ca. Die zweiwertigen Kationen sind gegen die einwertigen wie in der folgenden

Reihenfolge antagonistisch, bei Ca : Rb<Na<K<Li, bei Ba : Li<Rb<Na<K, bei Mg : Rb<Li<Na<K, bei Sr : Li<Ba<Na<K. Die antagonistische Wirkung eines Salzes gegen die anderen ist nicht nur von der Konzentration, sondern auch vom Verhältnis zweier Salzen abhängig. Die Wirkung der ein- bzw. zweiwertigen Kationen untereinander ist aus der Kolloidaktivität der betreffenden Ionen verständlich, sodass die Kationenreihe K<Na<Li<Rb bzw. Sr<Mg<Ba<Ca dabei für die Wirkungsweise ausschlaggebend ist, indem das Kation links in der Reihe beschleunigend, rechts hemmend wirkt. Hinzuzufügen ist, dass bei dem O-Verbrauch der Kalkfaktor eine grosse Rolle spielt.

65. On the Effect of a Centrifugal Force upon the Egg Cell and Proembryo of *Pinus Thunbergii* PARL. with Some Observations on Various Effects of Fixing Agents in the *Pinus* Egg Cell. T. SHIMAMURA. (Cytologia **1**, 1929, 59–66, 2 pls. and 1 text-fig.).

The mature cones of the previous year's growth of *Pinus Thunbergii* which were collected in June, were subjected to the centrifugal force by the rotation for one hour at a speed of 2500–3000 revolutions per minute at the radius of 15 cm.

The nucleolus in the egg nucleus is relatively much heavier than the other constituents of the nucleus, so that by centrifuging it is driven away into the end of centrifugal direction, and may often be forced out through the nuclear membrane into cytoplasm. The proteid vacuoles having the same density as the cytoplasm where they are imbedded, are not thrown into clearly defined zones by centrifuging. In the early proembryo stage where 2 or 4 free nuclei are found above the centre of the egg cell, they are not dislocated by centrifugal force, but in the proembryo stage, after these nuclei have reached the bottom of the egg cell, they can be easily moved towards the centrifugal end through the general cytoplasm of the egg cell. The comparison of these contrary states of things indicates according to the author the decrease of the density of the egg cytoplasm rather than the increase of that of the embryonal nuclei.

66. Über die Chromosomenzahl bei einigen Potentillen. Naomasa SHIMOTOMAI. (The Sc. Rpts. Tôhoku Imp. Univ. 4th Ser. (Biol.) **4**, 1929, 369–371, 2 Abb.).

Die haploide Chromosomenzahl bei *Potentilla chinensis* beträgt 7. Die somatische Chromosomenzahl bei zwei anderen Arten beträgt 14 (somit diploid wie bei *P. chinensis*) und bei zwei anderen Arten 28 (tetraploid).

67. On the Tetrapartite Chromosome in *Humulus Lupulus*. Yosito SINOTÔ. (Proc. Imp. Acad. **5**, 1929, 46–47, 4 figs.).

Contrary to the observation of WINGE who has reported in 1923 the presence of a distinct sex-chromosome pair of XY-type in *Humulus Lupulus* the author has observed in the same species besides 8 autosomic gemini one tetrapartite chromosome, which according to him, should represent the sex-chromosome of a new type. (Cf. No. 68).

68. Chromosome Studies in Some Dioecious Plants, with Special Reference to the Allosomes. Yosito SINOTÔ (Cytologia, **1**, 1929, 109–191).

The chromosomes of the male plants of 17 genera, 22 species and two varieties of dioecious phanerogams have been investigated. Of these the following 13 forms show

each an unequal pair of chromosomes in addition to autosome pairs at the meiotic division in microsporocytes. This unequal pair of chromosomes is assumed to be a sex chromosome complex of an XY-type. The 13 forms are *Salix leucopithecia*, *S. sachalinensis*, *S. japonica*, *S. melanostachys*, *S. gracilistyla*, *S. viminalis* var. *yezoensis*, *Morus bombycis*, *Cannabis sativa*, *Datisca cannabina*, *Daphniphyllum macropodum*, *Trichosanthes japonica*, *Hydrilla verticillata* and *Trachycarpus excelsus*.

All *Salix* plants studied, except one form of *S. sachalinensis*, have 19 as the gametic chromosome number which is basic in Salicaceae. The meiotic division is quite normal in them. One form of *S. sachalinensis* from Hokkaidô has ca. 24 chromosomes at the first meiotic metaphase, and their behaviour in the meiotic stages is irregular.

Humulus japonicus, growing wild in the vicinity of Tokyo, has a tripartite chromosome in addition to 7 autosomic gemini at the first meiotic division in microsporocytes. At the first meiotic metaphase, the tripartite chromosome divides in such a way that the two end chromosomes go to the one pole, while the middle one goes to the other. As a result, with respect to chromosomes, two kinds of pollen grains may be formed.

Humulus lupulus has 10 gametic and 20 zygotic chromosomes. In the first meiotic division of microsporocytes, 16 chromosomes out of the 20 form 8 gemini in all, while 4 remaining chromosomes do not form gemini, but are connected end to end to form a beaded string. This tetrapartite can be identified in several stages from early diaphase in the first meiotic division. At metaphase each alternate chromosome of the tetrapartite goes to opposite poles respectively. Thus the daughter nuclei receive an equal number of chromosomes, i.e. 10 respectively. The two middle members of a tetrapartite chromosome are equal in size and larger than the two end ones which differ in size from each other. As a result, two kinds of gametes may be formed, one having a larger amount of chromatin volume than the other. The tetrapartite chromosome may be a sex chromosome complex in *H. lupulus*, a new type of sex chromosome. (Cf. No. 67.—Ed.)

In regard to chromosomes, *Hydrilla verticillata* contains at least two forms. The one has 8 gametic and 16 zygotic chromosomes, while the other has 24 zygotic ones. In both forms an unequal chromosome pair is found in meiotic phase of microsporocytes. The second form is probably tribasic, derived from the union of a monobasic and a dibasic gamete.

To see whether the karyo-constitutional digamety of pollen grains is displayed in external character, the size of the pollen grains of some dioecious plants, whose digamety was proved cytologically, was measured. In *Cannabis sativa* and *Rumex acetosa*, the curves of size variation of pollen grains are bimodal. In *Humulus japonicus*, 1070 pollen grains were measured. The curve thus obtained is monomodal. From these results, together with other authors' results in other species, no definite conclusion can be drawn concerning the point in question.

Germination experiments were undertaken on pollen grains of *Rumex acetosa*, *Cannabis sativa* and *Humulus japonicus*. The results obtained in *Cannabis* and *Humulus* show that the size of pollen grains does not probably materially affect their

germination ratio. It is difficult, therefore, to know which of the different sized pollen grains are male or female determiners. Author.

69. Über den Wechsel des elektrischen Leitungsvermöigens bei den Bäumen. (Japanisch). Seitaro SUZUKI und Hukuyoshi OOMORI. (Mitteil. aus 1928 zu Hukuoka gehaltenen Wiss. Ver. in Japan **4**, 424–429, 3 Abb.)

Experimente wurden ausgeführt, um den Wechsel des elektrischen Leitungsvermögens bei einigen Pflanzen (z. B. *Pinus*, *Eucalyptus*, *Colocasia*, *Pueraria*) nachzuweisen. Die als Elektroden benutzten Insektennadeln wurden so tief im Pflanzenkörper eingesteckt als sie durch das Cambium bis zum Holzteile reichen. Die elektrische Druck war 1,4–2 Volt stark (trockene Batterien), und man hat die Experimente so ausgeführt, dass die Kurve für den Wechsel des hauptsächlich in der Längsrichtung des Pflanzenkörpers verlaufenden Stromes auf das Bromsilberpapier automatisch gezeichnet wird. Unter einer Anzahl von Experimenten ist ein hervorzuheben, wobei ein frischer Zweig von *Pueraria triloba* im Glasrohr eingeschlossen ist und die in seinem Innern herrschende Temperatur künstlich verschiedenartig verändert und der dazu entsprechende Wechsel des Leitungsvermögens bestimmt wurde. Es wurde dabei festgestellt, dass die Kurven für den Wechsel der Temperatur und des Leitungsvermögens fast gleichartig sind, woraus die Verf.n zum Schlusse angekommen sind, dass der Wechsel des elektrischen Leitungsvermögens im frischen Holz in erster Linie physikalisch bedingt wird, d. h. die Zunahme der durch die Temperatur verursachten physikalisch-chemischen Dissoziation der Elektrolyten im Pflanzensaft dabei eine grosse Rolle spielt.

70. Ovogenesis in *Coccophora Langsdorffii* (TURN.) GREV. Masato TAHARA. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1927, 551–556, 1 pl. and 2 text-figs.).

The oogonium of *Coccophora Langsdorffii* becomes 8-nucleate on account of three successive nuclear divisions. Of these eight nuclei one is found at the centre of the oogonium, and the other seven which come to its periphery degenerate. The chromosome number is 32 in the heterotypic, and nearly 60 in the somatic division. The centrosome is not distinctly seen.

71. On the Inheritance of Some Characters in *Glycine Soja*, BENTHAM (Soy-bean). Fumi TAKAGI. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**. 1929, 577–589, 1 pl. and 2 text-figs.).

The crossing between two forms of soy-bean, viz. Keitômame and Kurakake was performed. In the former stem is fasciated, flower white, hilum brown, and seed has no pattern. In the latter stem is normal, flower purple, hilum black, and seed has saddle-pattern. Normal stem, purple flower, black hilum, and no saddle-pattern are dominant to fasciated stem, white flower, brown hilum and saddle-pattern respectively. The segregation goes in the monohybrid way, except in the last character, which shows the dihybrid segregation. No linkage between the colour and pattern characters of seeds was detected. Green leaves are dominant to chlorotic, and the segregation goes in dihybrid fashion.

72. Fossile Koniferenhölzer aus Sendai-Tertiär I. Masahiko TAKAMATSU. (The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 533-542, 3 Tafeln u. 4 Textfig.).

In der Nähe von Sendai ist die tertiäre Braunkohlenlager weit verbreitet. Der Verf. hat in der vorliegenden Mitteilung die anatomischen Studien einiger in solcher Lager befindlichen fossilen Hölzer veröffentlicht, nämlich *Taxodioxylon sequoianum* (MERCKL.) SCHMALH. erw. GOTHAN em., *T. ishikuraense* sp. nov. und *Cupressinoxylon thuyopsoides* sp. nov. Betreffend jede Art ist die anatomische Struktur ausführlich beschrieben.

73. Stimulating Action of Oxyphthalein Colouring Matters on the Geotropism in Rice-Seedling with Special Reference to its Effect on the Growth of Length. Hatiwo TAKEDA. The Sc. Rpts. Tôhoku Imp. Univ. IV. Ser. (Biol.) **4**, 1929, 557-576, 6 figs.).

Among oxyphthalein colouring matters bromides or iodides confuse the geotropism in rice-seedlings by their dark action, which may be said to be roughly proportional to the intensity of fluorescence produced by the water solution of each dye in light. Dyes which are iodides disturb mainly the negative geotropism of the plumule, while those which are bromides disturb the positive geotropism of the radicle. The cause of disturbance is due to the existence of iodine or bromine which combines with the resorcinol-nucleus of these dyes.

74. Karyological Studies in *Hemerocallis*. Y. TAKENAKA. (Cytologia **1**, 1929, 76-83, 2 pls.).

The author, by examining the pollen mother-cells and the root-tips of 10 species of *Hemerocallis*, has found in 7 out of them n=11 and 2n=22, while in 2 others (*H. fulva*, *H. disticha* var. *Kwanso*) n=11-20, 3n=33, and in another one also 3n=33 (triploid!) were seen. 7 species above mentioned with n=11 and 2n=22 are quite fertile. Other 3 (triploids) are sterile. Their pollen grains can well germinate, but do not function, which owes no doubt to the disturbance caused by irregular meiotic division. Their embryo-sac may also degenerate on account of the irregular meiosis and cause sterility.

75. Investigations on the Relation between Plants and Their Surrounding Conditions by the Quantitative Method. III. A Comparative Study on the Various Kinds of Plants under the Potometer Condition; on Their Transpiration, Water-absorption and the Interrelation between two Preceding Functions. (Japanese with English résumé). Makoto TAKENOUCHI. (Bult. Sc. Fak. Terk., Kjusu Imp. Univ. **3**, 1929, 263-286).

The water relation indicated by the transpiration and the water-absorption were studied on 33 species of herbs of different oecological habits under the potometer condition for the first 24 hours.

When well-water was used, it was found that the ratio A/T (absorption to transpiration) was about 1 in the mesophytes, much greater than 1 in the halophytic succulents, and somewhat larger than 1 in the non-halophytic succulents. When seawater is used, A/T is smaller than 1 in the mesophytes, much larger than 1 in the

halophytic and intermediate in the non-halophytic succulents. The mesophytes become then much wilted, while the halophytes do not. The non-halophytic succulents, though they do not become wilted, exhibit evident signs of disturbance of internal tissues. From the latter fact we may see that there is a difference between halophytic and non-halophytic succulents concerning the resistance against sea-water.

76. Studien über die Stoffwechselphysiologie von *Aspergillus oryzae*. III. Mitt. Hiroshi TAMIYA. (Acta Phytochimica, **4**, 1929, 227–295).

Der Verfasser konstruierte eine bequeme Versuchseinrichtung für die genaue volumetrische Messung der Schimmelpilzatmung. Die Sauerstoffatmung und das Wachstum von *Aspergillus oryzae* gehen am lebhaftesten vor sich bei einer Gasmischung von etwa 17 Teilen N_2 und 83 Teilen O_2, und nehmen desto stärker ab, je mehr die Zusammensetzung des Gasmediums von diesem Verhältnis abweicht. Bei der Entziehung des Zuckers aus der Kulturlösung erleiden sowohl das Pilzwachstum als auch die Intensität der Atmung und der Gärung eine starke Herabsetzung. Eine deutliche Hemmung des Wachstums sowie der Sauerstoffatmung und der Gärung findet bei Zugabe von Kaliumcyanid statt, wobei aber die CN-Empfindlichkeit der Gärung im Vergleich mit derjenigen der beiden anderen Vorgänge am geringsten ist. Hemmt man die Sauerstoffatmung des Pilzes durch O_2-Entziehung, so steigt die Gärungsintensität in einem bestimmten Verhältnisse an. CN-Zugabe führt auch eine analoge Erscheinung herbei, vorausgesetzt, dass die CN-Menge nicht gross genug ist, um auch die Gärung stark zu beeinträchtigen.

Kohlenoxyd wirkt sehr stark schädigend auf den Aufbaustoffwechsel des Pilzes, und zwar ganz deutlich schon bei 10%-iger Zugabe. Gegen die Sauerstoffatmung und die Gärung wirkt aber Kohlenoxyd gar nicht hemmend. Wenn bei CO-Gabe öfters die Verminderung der absoluten Grösse des Sauerstoffverbrauches beobachtet wurde, dann ist sie immer auf die erhebliche Massenverringerung des aktiv atmenden Versuchsobjektes zurückzuführen. Auffallenderweise scheint bei hinreichender O_2-Zufuhr die Intensität der Atmung sowie der Gärung durch Einwirkung von Kohlenoxyd sogar vergrössert zu werden.

Der Oxydationsquotient betrug unter normalen Kulturbedingungen durchschnittlich 0.5–1.0, also etwa 1/6 von den Werten, die von MEYERHOF bei Muskeln und Hefe gefunden waren. Die Ursache hierzu sieht der Verfasser darin, dass bei diesem rasch wachsenden Pilze ein viel grösserer Teil der durch die Sauerstoffatmung gewonnenen Energie zum Aufbaustoffwechsel verwandt wird als bei der Hefe. Obwohl dieser Wert unter normalen Kulturbedingungen recht konstant ausfällt, nimmt er bei Zuckerentziehung oder bei CN-Zugabe mehr oder minder zu, aber bei CO-Zugabe immer deutlich ab. Diese Verschiedenheit wurde auch auf die dabei stattfindende Modifizierung der Energieverwendung zum Aufbaustoffwechsel zurückgeführt.

Zwischen der Sauerstoffatmung und der Wachstumsfähigkeit besteht fast immer eine innige Beziehung. Der Aufbauquotient ($AQ=\frac{\text{Pilzgewichtszunahme in g}}{\text{veratmeter Zucker in mg}}$) beträgt unter normalen Kulturbedingungen 0.4–1.0. Bei der Zuckerentziehung oder CO-Zugabe nimmt dieser Quotient immer deutlich ab, während er bei teilweiser Hemmung der Sauerstoffatmung durch O_2-Mangel oder CN-Zugabe mehr oder minder zunimmt.

Verf.

77. Zur Kenntnis der Dehydrase und des Glutathions in Schimmelpilzzellen. Hiroshi TAMIYA. (Acta Phytochimica, 4, 1929, 297–311).

Das Mb-Reduktionsvermögen und der Gehalt an Glutathion wurden an der Kultur von *Aspergillus oryzae* untersucht. Aerobe Decken zeigen in weiten Grenzen unabhängig von dem Alter der Kultur, und zwar selbst nach 38-tägigem Stehenlassen, noch ein starkes Reduktionsvermögen gegen Methylenblau. Kulturlösungen, die von den Pilzmyzelien vollständig befreit wurden, bewirkten auch eine starke Reduktion des Methylenblaus. Dieses merkwürdige Vermögen der Kulturlösung wird aber bei veralteten Kulturen stark abgeschwächt. Durch Anaerobiose wird die Kraft der Methylenbaureduktion in der Decke etwas herabgesetzt; sie behält aber sehr lang eine gewisse Stärke bei. Der durch das Auswaschen der Decke erhaltene Rückstand zeigt eine geschwächte Mb-Reduktion, während die durch Kochen hergestellten Auszüge der Decke gar kein Reduktionsvermögen zeigen. Beim Zusammenbringen dieser beiden Faktoren entsteht aber eine normale Reduktionskraft. Die grösste Reduktionsgeschwindigkeit beobachtet man bei Zusammenbringen der nichtbehandelten Decken und Kulturlösungen.

Die Pilzdecke zeigt im grossen und ganzen denselben Glutathiongehalt wie Hefe und Muskeln c. 0.2–0.5% bezogen auf Trockengewicht, 0.04–0.1% bezogen auf Rohgewicht. Der Glutathiongehalt der aeroben Pilzdecke ist desto grösser, je jünger die Kultur ist, und nimmt allmählig ab, um nach 5-monatelangem Stehenlassen gänzlich zu verschwinden. Bei der Anaerobiose zeigt die Glutathionmenge in der Pilzdecke eine geringe Abnahme, die aber bei langer Kulturdauer nicht wesentlich weiter geht, sodass selbst nach 5 Monaten Nitroprussidreaktion noch deutlich bemerkbar ist. Das von der Decke gebildete Glutathion wird auch allmählig in die Kulturlösung ausgeschieden. In aeroben Kulturen zeigt der Glutathiongehalt der Kulturlösung bei einem gewissen Stadium einen maximalen Wert, sinkt aber dann nach und nach herab, um schliesslich gänzlich zu verschwinden. Bei der anaeroben Kultur verbleibt das Glutathion sehr lang in der Kulturlösung, vielleicht in einem gewissen Gleichgewicht mit demjenigen in der Pilzdecke.

Der Verfasser hat schliesslich die Frage nach der physiologischen Bedeutung des Mb-Reduktionsvermögens und des Glutathions in Bezug auf die aerobe sowie anaerobe Atmung diskutiert und diese in mehr negativem Sinne beantwortet. Verf.

78. Über den Einfluss des Kohlenoxyds auf den Stoffwechsel des Schimmelpilzes. Hiroshi TAMIYA. (Acta Phytochimica, 4, 1929, 313–326).

Der Einfluss des Kohlenoxyds auf das Wachstum und die O_2-Atmung von einem typischen Schimmelpilz, *Aspergillus oryzae*, wurde näher untersucht, und zwar mit derselben Versuchseinrichtung wie sie in der vorstehend referierten Arbeit von demselben Verfasser angewandt wurde. Der Pilz atmet in den Gasmischungen 50% CO+50% O_2 und 70% CO+30% O_2 keineswegs mit geringerer, sondern sogar mit etwas grösserer Intensität als in der Gasmischung 50% N_2+50% O_2 bzw. 70% N_2+30% O_2. Die Gasmischung 90% CO+10% O_2 bewirkt eine etwa 33%-ige Herabsetzung der Atmungsintensität der Pilzdecken im Vergleich mit 90% N_2+10% O_2. Nach 4-stündigem Aufenthalt in diesen Gasmischungen wurden beide Decken in der Atmosphäre 85% O_2+15% N_2 weiter kultiviert. Die mit CO vorbehandelten Pilzdecken zeigten dabei im Vergleich mit derjenigen der Kontrollkultur eine 79%-ige Herab-

setzung der Wachstumsfähigkeit, während ihre Atmungsintensität um etwa 20% gesteigert wurde. Einige Pilzdecken wurden in 90% CO+10% O_2 und andere in 100% N_2 gebracht, und nach 3–4 Stunden wurden die Gase gleichfalls durch ein Gasgemisch 85% O_2+15% N_2 ersetzt. Die ersteren Decken zeigten dann gegenüber den letzteren eine 57%-ige Herabsetzung der Wachstumsfähigkeit, aber sogar 11%-ige Steigerung der Atmungsintensität. Die 3 Stunden mit 50% CO+50% O_2 behandelten Pilzdecken zeigen beim Versetzen in 85% O_2+15% N_2 eine stärkere Atmungs- sowie Wachstumsfähigkeit als die mit 100% N_2 vorbehandelten Decken. Die mit 70% CO+30% O_2 vorbehandelten Decken zeigten dabei eine 19%-ige Wachstumsabnahme, aber eine 26%-ige Atmungszunahme. Auf Grund solcher Tatsache hat der Verfasser den Schluss gezogen, dass unter Einwirkung von grösseren Gaben des Kohlenoxyds, welches an sich selbst die Atmung gar nicht hemmt, die Lebenstätigkeiten des Schimmelpilzes mehr oder minder stark beeinträchtigt werden, und dass ferner die bei Anwendung sehr grosser Mengen von Kohlenoxyds zur Beobachtung kommende Atmungshemmung bei Schimmelpilzen nur eine scheinbare und sekundär durch die erwähnte Zellbeschädigung hervorgerufene Erscheinung darstellt. Verf.

79. Bibliographie von *Aspergillus* 1729 bis 1928. Hiroshi TAMIYA und Shinkichi MORITA. (Bot. Mag. Tôkyô **43**, 1929, 321–332, 371–381, 427–438, 501–515, 577–589, 627–633).

Fortsetzung des im Titel genannten Literaturverzeichnisses. Beginnt mit Nr. 740 (1903) und endet mit Nr. 1655 (1920). Fortsetzung folgt.

80. On the Distribution of *Citrus* and *Citrus* Relatives. (Japanese with English résumé). Tyôzaburô TANAKA. (Studia Citrol. **3**, 1929, 22–32).

The plants belonging to the subfamily Aurantiae are mostly of the East-Asiatic origin, though partly of the African, never of the American. The genus *Citrus* occurs from India to Malay Archipelago. According to the author the Central China and Himalayan region are to be regarded as the most important homes of many different groups of *Citrus* and near genera.

81. *Chalcas*, a Linnean Genus which includes Many New Types of Asiatic Plants. (Revisio Aurantiacearum IV.) (With Japanese résumé). Tyôzaburô TANAKA. (Jour. Soc. Trop. Agric., Taihoku Imp. Univ., Taiwan **1**, 1929, 23–44.)

Species and varieties of the genus *Chalcas* which was described in 1767 by LINNAEUS and four years later renamed *Murraya* by him, are described, of which some are new.

82. Vererbung der in der heterotypischen Kernteilung gebildeten Chromosomenringe bei *Oenothera*. (Japanisch). Moriki TUDA. (Japan. Jour. Gen. **4**, 1929, 115–116).

Der Verf. hat die Chromosomenringbildung bei der heterotypischen Kernteilung von *Oenothera Lamarckiana*, *biennis*, *biennis cruciata*, *sinuata* und bei 5 verschiedenen F_1-Hybriden zwischen den obigen Arten studiert. Die Resultate sind wie folgt:

Oenothera Lamarckiana (12)+2 (die in Parenthesen eingeschlossenen Ziffern bedeuten die Zahl der zu einem Ringe vereinigten Chromosomen); *O. biennis* (6)+(8), *O. biennis*×*cruciata* wie *biennis*, *O. sinuata* (14), *Lamarckiana*×*biennis cruciata* (12)+2, *biennis*×*biennis cruciata* (6)+(8), *sinuata*×*Lamarckiana* teilweise (10)+(4), *biennis*×*sinuata* (6)×(8), *sinuata*×*biennis* (14), *biennis*×*Lamarckiana* (6)+(8), (12)+2 usw.

83. Variation of Chromosome Number among F_2- and F_3- Progenies in the Crosses between two Dwarf Wheat Plants. Shunjiro WAKAKUWA. (Japan. Jour. Gen. **4**, 1929, 187–197, 1 pl.)

35 somatic chromosomes possessed by the pentaploid hybrid, *Triticum ponticum*× *T. Spelta* are composed of 14 bi- and 7 univalents. The former consist of 14 *polonicum*- and 14 *Spelta*-chromosomes, and the latter of the remaining 7 *Spelta*-chromosomes. Though 14 bivalents behave quite normally in the homoeotypic division, 7 univalents are then distributed at random to either pole, so that in pollen-grains and egg-cells the chromosome number is to be represented by the formula 14+i, i varying from 0 to 7. These 7 chromosomes are called a, b, c, d, e, f and g respectively. In the F_4- and F_5-Generation of the pentaploid hybrids KIHARA obtained two dwarf plants $20_{II}+0_I$, one of them being 2 (14+a+b+c+d+e+f), i.e. without g, the other 2 (14+a+b+c+d+e+g), i.e. without f.

The present paper refers to the crossing experiments of these two dwarf plants. In F_1 there are besides 19 bivalents 2 univalents which are evidently f and g. The bivalents behave normally. The univalents divide equationally in the heterotypic division, but are distributed to both poles at random in the homoeotypic division, so that the gametic chromosome number varies from 19 to 21. In the offspring of 39-chromosomic plants ($19_{II}+1_I$), individuals, such as $19_{II}+0_I$, $19_{II}+1_I$, $20_{II}+0_I$ are observed, from which we see that the pollen-grains having 19 and 20 chromosomes are effective. All offspring from the plant 19_{II}+f and those from 19_{II}+g lack g- and f-chromosome respectively. The former are more highly sterile than the latter, which indicates that the degree of sterility is due partly to the difference of the lacking chromosome.

84. On the Influence of Gravity upon the Development of Embryo of *Pinus Thunbergii* PARL. K. WAKAYAMA. (Cytologia **1**, 1929, 68–75, 1 pl. and 1 fig.).

The experiments on *Pinus Thunbergii* were so arranged that the branches were bent by certain contrivances, so as to raise the hanging strobilus attached to them vertically upwards. The observations were made on materials collected at several intervals and fixed according to usual methods.

The orientation of the nucleus within the egg cell is not disturbed by the inverted position of the cone, and is therefore independent of the action of gravity. In treated material the nucleolus was always found close to the micropylar wall of the nucleus, while in the control it was placed on its antimicropylar wall. This is in accord with some observation in animal eggs, where the nucleolus was found in contact with the lowest part of the nuclear membrane.

The first mitotic figure after fertilization is almost always oblique to the vertical axis of the egg cell, as it is usually the case, but in the later stage of mitosis the mitotic figure tends to rotate until its major axis comes to lie transversally to the long

axis of the egg, so that the two resulting daughter nuclei lie side by side. In the second mitosis the same phenomenon may be observed, while in the succeeding mitoses no such thing takes place, which is therefore independent of the action of gravity. Further processes in the formation of the proembryo, for instance, the migration of four free nuclei towards the base of the egg, the direction of the growing suspensor, etc. go quite in the same manner in normal as well as treated materials, and therefore such processes should be also independent of the action of gravity.

85. Über die Absorption des Harnstoffes durch den Pflanzenkörper und seinen Nährwert als Stickstoffquelle. (Japanisch). Sennosuke YAMAGUTI. (Mitteil. landw.-forstl. Ver. Sapporo **21**, 1929, 58 S.).

Einige Ergebnisse des Verf.s experimentellen Untersuchungen sind wie folgt. Es wurde durch die Methode der Wasserkultur die Tatsache nachgewiesen, dass die jungen Maispflanzen den Harnstoff als solchen absorbieren. Der letztere bleibt zuerst als solcher im Pflanzenkörper während einiger Zeit, dann erst darin bewegt, um als N-Quelle zu dienen. Die absorbierte Menge ist natürlich von seiner Konzentration in der Nährlösung abhängig, doch schon bei 0,1 Mol. wirkt er auf das Pflanzenwachstum etwas schädlich, besonders wenn die Mikroorganismen darin vorhanden sind, wegen der Ammoniakbildung. Der Harnstoff entspricht in seinem Nährwert dem Ammoniakbikarbonat, welches eine ihm entsprechende N-Menge enthält. Urease ist nur im Embryo sowie dem Skutellum der jungen Maispflanze nachzuweisen und nichts andererorts.

86. Further Contributions to the Knowledge of the Second (S–M–) Linkage Group in Rice. (Japanese). Yasuke YAMAGUTI. (Nôgakukenkyû (Agr. Research), **13**, 1929, 135–172).

With regard to the second (**S–M–**) linkage group, the author reports the results obtained in 1927–1928. In two crosses the average shooting time for each individual is calculated from the results of respective individuals in the F_3 generation. Between the empirical and the calculated shooting time strong correlations were found: $r=0{,}803$ in one case, $r=0{,}734$ in the other. The genes which were analysed were: $\mathbf{F_1}$, $\mathbf{F_2}$, $\mathbf{F_x}$: shooting time early or late; **M**: endosperm starchy or glutinous; $\mathbf{R_1}$: pericarp red or white; **S**: apiculus coloured or colourless.

The crossing-over percentage in two crosses, having one parent in common, on the basis of calculated shooting time were found to be: for $\mathbf{F_1}$–**M**: 13,6% resp. 8,8%. The individual shooting time observed in the F_2 generation, computed in linkage to **S** or **M**, gives lower crossing-over percentages than the calculated. In the F_3 generation were found: for $\mathbf{F_1}$–**M** 15,6%; for $\mathbf{F_1}$–**S** 16,5%; for **M**–**S** 22,6% in repulsion phase, 15,7% in coupling phase. When another gene for shooting time ($\mathbf{F_2}$) apparently entered into the cross in heterozygotic representation no linkage with **S** or **M** was found in the case where the gene $\mathbf{F_1}$ is in homozygotic representation, while in the case of its heterozygotic representation the linkage between $\mathbf{F_1}$ and **M** proved to be very weak (39,5% resp. 31,3% crossing-over).

In the F_2 generation of other crosses were found: for **M**–**S** 20% resp. 33% in two cases (the latter deviation from the former was caused by the irregularity of segregation of S); for $\mathbf{F_1}$–**M** 20, 4%; for $\mathbf{F_1}$–**S** 20%.

The F_2 results of another cross, where one parent belonged to the race with red grain, showed a new correlation between $\mathbf{R}_1$ and $\mathbf{F}_x$, which if computed as a true linkage, corresponds to the one with 22,1% crossing over. $\mathbf{R}_1$ has no apparent correlation with **S** or **M**. Here also **M**–**S** is linked with 20,3% and $\mathbf{F}_x$–**M** with 36,9% crossing-over, while for **S**–$\mathbf{F}_x$ some 50% crossing-over was found. So the idea of **S**–**M**–$\mathbf{F}_x$ order of respective genes is justified. The nature of $\mathbf{F}_x$ remains to be studied further. The gene for shooting time in coexistence with **m** (glutinous endosperm) was so easily changeable by the environment, that in place of repulsion an unexpected coupling phase resulted. Author.

87. Genetical Studies on Rice. (Japanese). Yasuke YAMAGUTI. (Annual Rep., Saitô Hô-on Kwai **5** for the year 1928, 1929, 136-139).

Together with the **S-M-**$\mathbf{F}_1$ linkage group and the new correlation between $\mathbf{R}_1$ and **Fx** (see Abstract No. 86) are reported one segregation of the gene for tall or dwarf habit, independent from the **S-M-**$\mathbf{F}_1$ linkage group and the trihybrid segregation of the colour of leaf and stigma, although the relation of their genes to those of the **S-M-**$\mathbf{F}_1$ linkage group remains undetermined. A case of semi-sterility (25–35% fertility), resulting from crossing, is reported. In its F_2 generation the segregation into fertile and semi-sterile ones was evident. Owing to the small number of individuals the genetical analysis of this phenomenon remains not yet completed. Author.

88. The Variation and Correlation among Varieties of Wheat and Barley in Regard to the Resistance to the Toxic Action of Potassium Chlorate. (Japanese with English résumé). Morimasa YAMASAKI. (Jour. Imp. Agric. Exp. Sta. **1**, 1929, 139-162).

The toxic action of $KClO_3$ towards seedlings of wheat and barley was studied by the application of 0.605–0.1% solutions during 48 days. Naturally various grades of resistance against toxic action was distinguishable. It was found, firstly that the hardier in winter, the less is the resistance, secondly that the early maturing varieties are more resistant than the late maturing ones, and thirdly that barley varieties with naked kernels are more resistant than those with the ordinary ones. It was further ascertained that the seedlings grown in shade, those grown under high temperature and those grown with fertilizer are more resistant than those grown under respective reverse conditions.

No definite relation was found between the cell-sap concentration and the resistance. The solution absorbed in the culture with 0.1% $KClO_3$ and the water absorbed in the culture with distilled water are much larger in the more resistant varieties than in the less resistant ones, but the absorbed amount of the toxicant solution compared with that of distilled water was smaller in the former than in the latter. When the solutions of the same concentration are used in the culture, the residual solutions were found to be more concentrated in the case of more resistant seedlings than in that of those less resistant.

89. Studies on the Maternal Inheritance of Plastid Characters in *Hosta japonica* ASHERS. et GRAEBN. f. *albomarginata* MAK. and its Derivatives. Kono YASUI. (Cytologia **1**, 1929, 192–215, 2 pls, and 5 text-figs).

Hosta japonica f. *albo-marginata* has green leaves with yellowish white margins, though rarely the white tissue may extend up near to the midrib. Plastids are green,

yellow or colourless. The cells composing the marginal tissue of its leaves are various in respect to its plastid contents: they contain either one kind of them or the mixture of two or three kinds in various combinations. Some of their offspring receive only one kind of plastid, while others more than one, and according to this fact as well as to the arrangement of the white tissue in leaves the offspring may be distinguished into several kinds, as *albo-marginata, albo-chlorovariegata, medio-albinata,* etc. besides wholly green plants. The crossing experiments have proved the maternal inheritance of plastid characters. It may be added that unlike *Funkia ovata* studied long ago by STRASBURGER no apogamy does occur here.

The writer's conclusion about the transmission of plastids is that the variegated plants under discussion originate from a fertilized egg cell which had two or more different kinds of plastids transmitted from the embryo-sac mother-cell.

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Studies on the Genetics and Physiology of Self- and Cross-incompatibility in the Common Cabbage (*Brassica oleracea* L. var. *capitata* L.)

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With 3 Figures in Text

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Introduction

The phenomenon of self- and cross-incompatibility in plants has been studied so far by many authors, and much has been contributed recently to the progress of the study. I may refer especially to the genetic hypothesis propounded by EAST and MANGELSDORF (1925, 1926) for explaining the case of the genus *Nicotiana*, as having thrown the most brilliant light on the subject. It is evident, however, that there exist still, concerning various kinds of plants, a number of cases in which the genetic behavior of the phenomenon has not yet been lucidly interpreted. As to its physiology too, the concensus of opinion has not been obtained seemingly on some points. The study under consideration will be the matter of the utmost importance, not only in plant biology but also in the practice of plant breeding and seed or fruit growing.

Experimental works on self- and cross-incompatibility have been carried on by the present author since 1922 with a certain number of cruciferous plants. At first, he tested the general status of fruit and seed setting in self-pollination, and found that in them highly self-incompatible individuals occur rather usually (KAKIZAKI, 1923, 1925). Then, he attempted to inquire the fundamental cause of the phenomenon. For this purpose the common cabbage, *Brassica oleracea* L. var. *capitata* L. was used. In view of the author's results of experiments above stated this plant is to be considered to be the most convenient material among the crucifers: so many flowers are generally produced on one individual that they can be used quite freely for various matings of pollination, and besides the flowers and flower stalks are substantial enough in constitution to make experimental manipulations quite easy. In the present paper the author intends, besides reporting the results of the studies, to offer a new genetic hypothesis as well as a new physiological interpretation of the phenomenon in question.

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I. Genetic Studies

1) Material and Methods

In 1924, a certain number of families of the variety Succession of the common cabbage were grown for the purpose of practical breeding.

From some of these families ten plants were chosen out in the spring of 1925, and ten plants of another variety Toyodawase were also selected out in the spring of 1928. On these twenty plants as well as their offspring, selfing and crossing experiments were carried out from 1925 to 1929.

Special attention was paid to the uniformity in vigor of the inflorescences within one and the same plant used for the experiment. Healthy flowers only which were situated in the middle part of each inflorescence were taken for use, unused flowers and flower buds then being cut off. Before any of the selected flowers opened, each inflorescence was covered with a paraffined paper bag.

The flowers used as females were castrated one or two days before blooming. In the 1925 experiment, however, the castration was not practised. This omission was done in the idea that the own pollen of the pistillate flower might not much disturb the result of its cross-pollination, because, so far as the author was aware, no instance had been reported that the fertility in self-pollination was higher than that in cross-pollination on the same plant. This remark is important for drawing an inference from the experimental result in 1925 which will be described later (s.p. 136).

The bagged flowers, when opened, were artificially pollinated with their own pollen (selfing) or with pollen of a bagged inflorescence of another plant (crossing). About ten days after pollination, the bag was removed and unhealthy flowers, if any, were discarded. In studying the fertility in this plant the performance of experiments was made much easier than otherwise, owing to the fact that, at this stage, unhealthy flowers are quite easily distinguished from healthy ones, inasmuch as the pistils of the former turn yellowish while those of the latter, even when perfectly sterile, grow into small green pods.

In the studies on self- and cross-incompatibility made by many investigators, it seems rather usual that the fertility has been tested on the basis of fruit setting with no consideration at all about the number of seeds contained therein. In the present investigation, however, besides the number of fruits with and without seeds, the number of seeds in each fruit was counted. This counting does not want much labor on account of comparatively small number of seeds per fruit.

In Toyodawase the number of seeds per fruit seemed to be very variable in different plants, and therefore it was considered hardly possible to make accurate comparison of the grades of fertility of different plants even on the basis of the number of seeds yielded by a certain number of flowers. Consequently, in the experiments with this variety, the fertility under the

natural condition of each plant was also observed, and the percentage of the average number of seeds per fruit in experimental pollination in relation to that in the natural pollination on the same plant was computed. This percentage is named "relative fertility".

2) Experiments with the Variety Succession

a) *Experiment in 1925*

All ten plants of the variety Succession chosen out in the spring of 1925 were, on one hand, self-pollinated, and on the other, cross-pollinated reciprocally in all possible combinations. In Table I(1), the result is given. The average number of seeds per flower is summarized in Table II, where the figures in italics refer to self-pollination.

The result shows that there exists in most matings actually a clear distinction between two groups, compatible and incompatible, although incompatible matings are not always completely sterile. The fact is not very clear, to which class, either compatible or incompatible, the crossing Plant 2×Plant 8 should belong, the figure of fertility in this case being 11.3. It is not reasonable to put this mating into the compatible class, as its fertility is considerably lower than that in all other matings, where Plant 2 was used for the pistillate plant. Moreover, Plant 2 is evidently self-compatible, and it is very likely that the grade of fertility in this crossing was somewhat raised by self-pollination, because, as mentioned before, the flowers used were not castrated. Accordingly, it would be reasonable to include this mating under the incompatible class. All the other matings are divided into the two groups by the middle horizontal line in Table III. The result in Table II is represented in Diagram 1, in which the compatible mating is denoted by F and the incompatible by S, self-pollination being shown in italic type.

The results indicates that the ten plants tested are to be put into the five distinct classes A, B, C, D and E regarding self- and cross-incompatibility. In respect to these classes the following facts are to be mentioned:

1. In the classes A, B and C, all the plants are self-incompatible, and also incompatible when crossed within the same class; but they are compatible when crossed with any individual of different classes, except that C♀×A♂ mating is always incompatible.

2. In the classes D and E, just contrary to the classes above cited,

(1) All tables are put together in the Appendix at the end of the paper.

each of the plants selected out is self-compatible, yet cross-incompatible between them.

3. Crossing between any plant of A, B or C on the one hand and that of D or E on the other is always compatible.

The fact that the mating C♀×A♂ is always incompatible while its reciprocal is compatible was apparently not due to imperfections of sexual organs of the plants concerned, since either all the individuals of the class

Pollen plants

Pistillate plants: Class	Plant No.	1	3	4	7	9	5	10	6	2	8
A	1	*S*	S	S	S	S	F	F	F	F	F
	3	S	*S*	S	S	S	F	F	F	F	F
	4	S	S	*S*	S	S	F	F	F	F	F
	7	S	S	S	*S*	S	F	F	F	F	F
	9	S	S	S	S	*S*	F	F	F	F	F
B	5	F	F	F	F	F	*S*	S	F	F	F
	10	F	F	F	F	F	S	*S*	F	F	F
C	6	S	S	S	S	S	F	F	*S*	F	F
D	2	F	F	F	F	F	F	F	F	*F*	S
E	8	F	F	F	F	F	F	F	F	S	*F*

Diagram 1. Shows the result of self- and cross-pollinations in 1925.

C used as pistillate plants or all those of the class A used as pollen plants were entirely effective in the crossings with those of other classes. Exactly the same could be said about the case of the reciprocal crossings between D and E.

b) *Experiment in 1927*

The selfed seeds obtained in the 1925 experiment were sown in early summer of 1926, and the genetic behavior of the progenies thus grown was tested in the spring of 1927.

Either of the two self-compatible mother plants Nos. 2 and 8 threw the offspring containing both self-compatible and self-incompatible plants as shown in Table IV, and the ratio of both types is very near 1:1 as summarized in Table V. As shown in Table VI, among the self-incompatible

mother plants, No. 5 gave also both types in equal numbers, and all the others only self-incompatible descendants.

Table VII shows the result of cross-pollinations between different plants of the progeny of the self-compatible plant No. 2. All matings, either between different self-compatible plants or between self-compatible and self-incompatible were equally compatible without exception. Between different self-incompatible plants, however, some matings were incompatible while others compatible. Reciprocal cross-pollinations gave the same results in all cases.

Further, the descendant (self-incompatible) of No. 1 was crossed with some of both self-compatible and self-incompatible plants of the progeny of No. 2. As given in Table VIII, it proved to be compatible with all the self-compatible plants in both reciprocal crossings, but, with the self-incompatible ones, it was incompatible in some cases and compatible in the other cases.

3) Experiments with the Variety Toyodawase

a) *Experiment in 1928*

In the spring of 1928 the ten plants of the variety Toyodawase gave under the natural condition fertility as shown in Table IX. Its degree varies considerably in different plants, the number of seeds per fruit ranging from 5.6 to 22.5.

With these ten plants, both self- and cross-pollinations were made. The result is given in Table X.

The variation in the fertility in this case is comparatively wide, and compatible and incompatible matings are not so distinct as in the case of the variety Succession. There is, however, in each plant a boundary between fertility of relatively higher and lower grades. If the matings are classed into the two groups as in Table XI, the result may be represented as in Diagram 2. It is doubtful whether the mating No. 13×No. 19 belongs to the compatible group or to the incompatible one; but it may be considered to belong to the former so far as the fertility of the other matings on No. 13 is concerned.

Then, the result in that year becomes to be summarized as follows:

1. The ten plants tested are put into six classes; plants belonging to the classes A, B, C and D are self-incompatible but those belonging to the classes E and F are self-compatible.

2. In the classes A and B, all plants within the same class are cross-

incompatible to each other. Each of the other four classes contains only one plant, so that the cross-fertility within the same class is unknown.

3. In the classes A, B and C, all plants between different classes are cross-compatible to each other in both directions.

4. The plant of the class D is cross-compatible in both directions with any individual of the class B; but with any individual of the classes A and

Pollen plants

Pistillate plants: Class	Plant No.	11	12	20	13	14	17	19	18	16	15
A	11	*S*	S	S	F	F	F	F	S	S	F
	12	S	*S*	S	F	F	F	F	S	S	F
	20	S	S	*S*	F	F	F	F	S	S	F
B	13	F	F	F	*S*	S	S	F?	F	F	S
	14	F	F	F	S	*S*	S	F	F	F	S
	17	F	F	F	S	S	*S*	F	F	F	S
C	19	F	F	F	F	F	F	*S*	S	F	F
D	18	F	F	F	F	F	F	F	*S*	F	F
E	16	F	F	F	F	F	F	F	F	*F*	F
F	15	F	F	F	F	F	F	F	F	F	*F*

Diagram 2. Shows the result of self- and cross-pollinations in 1928.

C, it is compatible when used as female and incompatible when used as male.

5. The plant of the class E is incompatible with any individual of the class A when used as male, but the reciprocal crossing and the crossings in both directions with any plant of all the other classes are compatible.

6. The plant of the class F is incompatible with any plant of the class B when used as male, but the reciprocal crossing and the crossings in both directions with any plant of all the other classes are compatible.

b) *Experiment in 1929*

Selfed seeds obtained in the experiment in 1928 were sown early in July of the same year. No. 12 produced no adult plant owing to the small number of the selfed seeds, although the other nine plants gave a certain number of descendants which were used in an experiment of the spring of 1929.

The degree of fertility of all these plants under the natural condition is shown in Table XII. Its variation in that year is pretty wide just as in the preceding.

All the plants were tested as to their fertility in self-pollination. The results of these experiments are given in Table XIII–XXI, and variations in the relative fertility in Table XXII. In this case they are also somewhat wide, but the middle horizontal line of Table XXII may be considered to form a boundary between the two groups, self-compatible and self-incompatible.

All these results show that the most families consist of both types, self-compatible and self-incompatible in certain proportions, but a few families contain self-incompatible only. The number of individuals in the family No. 20 is only four and consequently too small to be judged whether the family was ordinarily the constant or the segregating one. The family No. 18 contains nineteen plants, among which no self-compatible individuals were observed. It is a noticeable fact that all the self-compatible plants as well as some of the self-incompatible ones segregated into both types, and no constant family of self-compatible plants was obtained while one (No. 18) of the self-incompatible plants bred true to type.

Cross-pollinations were also carried out. For such experiments, all the nine plants of the family No. 11 and ten plants of each of the families Nos. 15 and 18 were used(1). Among the plants of each of these families, mutual cross-pollinations were performed in all possible combinations. The results are given in Tables XXIII–XXV, and variations in the relative fertility are shown in Table XXVI.

It may be considered that the middle horizontal line of Table XXVI forms a distinct boundary between compatible and incompatible matings. In this case, however, the variations of the compatible matings are somewhat wider and more irregular than in the case of self-pollination shown in Table XXII. In this respect detailed discussion will be made later.

Then, the crossing result of the family No. 11 is represented in Diagram 3, and that of the family No. 15 in Diagram 4. All the matings in the

(1) As will be discussed later, the author considers two factor series, **S** and **T**, to be responsible for the inheritance of self- and cross-incompatibility in cabbage, and he estimated from the result in 1928 that the classes A, B and C in that year would be constituted of both heterozygous **S** and **T**, the classes E and F would be of hetorozygous **S** and homozygous **T**, and the class D would be of homozygous **S** and heterozygous **T**. As the representatives of the progenies of these three categories, the family Nos. 11, 15 and 18 were respectively selected out as the materials of the crossing experiments.

family No. 18 may be considered incompatible. A summary is as follows:

1. Family No. 11.—The plants used for the crossing experiment are put into four classes, A, B, C and D. In the classes A, B and C, all individuals are self-incompatible as well as cross-incompatible within the same class. Only the plant belonging to the class D is self-compatible. The members of the class A are, when used as females, incompatible with the members either of the class B or C, while all the reciprocal crossings are compatible. Between the classes B and C, all matings are compatible in both directions. The plant of the self-compatible class D is compatible with

Pollen plants

Pistillate plants: Class		A				B	C			D
	Plant No.	11-6	11-7	11-8	11-9	11-5	11-1	11-2	11-4	11-3
A	11-6	*S*	S	S	S	S	S	S	S	S
	11-7	S	*S*	S	S	S	S	S	S	S
	11-8	S	S	*S*	S	S	S	S	S	S
	11-9	S	S	S	*S*	S	S	S	S	S
B	11-5	F	F	F	F	*S*	F	F	F	F
C	11-1	F	F	F	F	F	*S*	S	S	F
	11-2	F	F	F	F	F	S	*S*	S	F
	11-4	F	F	F	F	F	S	S	*S*	F
D	11-3	F	F	F	F	S	F	F	F	*F*

Diagram 3. Shows the result of self- and cross-pollinatiins in the family No. 11 in 1929.

the members of the class A, when used as female, but incompatible in the reciprocal crossings; with the plant of the class B, just contrary to the upper case, incompatible when used as female but compatible in the reciprocal way; and with the individuals of the class C, compatible in both directions.

2. Family No. 15.—The plants used for the crossing experiment are grouped into three classes, A, B and C. In the classes A and B, all members are self-incompatible as well as cross-incompatible within the same class; while in the remaining class D, all individuals are self-compatible as well as cross-compatible within the class. Crossings between different classes are compatible in all ways.

3. Family No. 18.—All individuals are self-incompatible as well as cross-incompatible within the family in all ways.

Pollen plants

Pistillate plants: Class	Plant No.	A				B	C				
		15-1	15-3	15-4	15-6	15-9	15-2	15-5	15-7	15-8	15-10
A	15- 1	*S*	S	S	S	F	F	F	F	F	F
	15- 3	S	*S*	S	S	F	F	F	F	F	F
	15- 4	S	S	*S*	S	F	F	F	F	F	F
	15- 6	S	S	S	*S*	F	F	F	F	F	F
B	15- 9	F	F	F	F	*S*	F	F	F	F	F
C	15- 2	F	F	F	F	F	*F*	F	F	F	F
	15- 5	F	F	F	F	F	F	*F*	F	F	F
	15- 7	F	F	F	F	F	F	F	*F*	F	F
	15- 8	F	F	F	F	F	F	F	F	*F*	F
	15-10	F	F	F	F	F	F	F	F	F	*F*

Diagram 4. Shows the result of self- and cross-pollinations in the family No. 15 in 1929.

4) Existing Hypotheses of the Genetic Behavior

Since CORRENS (1912) first gave in his study of *Cardamine pratensis* a Mendelian interpretation of the problem of self-incompatibility, COMPTON (1913) and a number of other investigators have reported many important results of genetic studies on various species of plants. It is perhaps unnecessary, however, to cite here all of them, since one can find the collection of literature on this subject in a paper of EAST and PARK (1917) as well as in that of LEHMANN (1928).

Self-incompatible classes, in which the individuals are cross-incompatible within their own class but cross-compatible with all the members of any other were firstly found by DE VRIES (1906) in *Linaria vulgaris.* Thereafter extensive studies on the similar classes were made with the genus *Nicotiana* by EAST (1915, 1917, 1918, 1919, 1923), EAST and PARK (1917), ANDERSON (1924), and EAST and MANGELSDORF (1925, 1926, 1927). Very apparent cases of such intra-incompatible and inter-compatible classes were found also by BAUR (1919) in *Antirrhinum hispanicum,* by LEHMANN (1919, 1922; 1927) in *Veronica syriaca,* by TERAO (1923) and TERAO and U (1929) in *Petunia violacea,* by SHULL (1924) in *Bursa bursa-pastoris,* by KIKUTI (1927, 1929) in Japanese pears, and by many other workers in several fruit trees such as apples, pears, plums and cherries.

To explain this phenomenon, a hypothesis that certain oppositional factors which form a series of multiple allelomorphs control self- and cross-incompatibility has been advocated by LEHMANN (1919, 1922), PRELL (1921), EAST and MANGELSDORF (1925), FILZER (1926) and other authors, and this view seems to predominate in this line of study nowadays. The hypothesis of EAST and MANGELSDORF (1925), for example, which may be considered perhaps to be the representative one, is as follows: Multiple allelomorphs S_1, S_2 and S_3 affect the rate of growth of the pollen tubes through the stylar tissue. The style of a plant with the formula S_1S_2, for instance, inhibits the growth of the pollen tubes possessing either S_1 or S_2, but allows the penetration of the pollen tubes only possessing S_3. Thus the pollen-tube growth is inhibited always when the same factor is found both in the pollen and in the style.

The difference in fertility between the reciprocal crosses, as observed quite frequently in the present author's case, was reported early by GARDNER (1913) in sweet cherries, by CORRENS (1916) in *Linaria vulgaris,* by STOUT (1916) in *Cichorium Intybus,* by SIRKS (1917) in *Verbascum phoenicium,* and later by EAST and MANGELSDORF (1925) and BRIEGER (1927) in *Nicotiana,* by CRANE (1925, 1927) in plums, and by WELLINGTON (1927) in apples. In the common cabbage also, DETJEN (1927) observed the same phenomenon.

EAST and MANGELSDORF (1925) consider that homozygous plants such as S_1S_1, for instance, can be produced by pseudofertility in premature self-pollination of self-incompatible plants, and that the S_1S_1 plants may be fertilized with S_2 pollen of S_1S_2 plants but the reciprocal crossing will be fully incompatible. SIRKS (1926, 1927) supposes that, for instance, S_1 pollen is incompatible not only in the S_1 style but also in the S_2 style, while inversely S_2 pollen can function in the S_1 style. BRIEGER (1927) offers another hypothesis that the false fertility factor **P** is coupled with the sterility allelomorph **S**, and its influence is such that it cancels the influence of the allelomorph coupled with it if the plant is used as a female, while it has no influence upon sterility if the same plant is used as a male.

In regard to the relation between self-compatible and self-incompatible plants, the results of COMPTON (1912, 1913) in *Reseda odorata,* BAUR (1919) in *Antirrhinum,* EAST (1919) and BRIEGER (1927) in *Nicotiana,* and TERAO (1919) in *Petunia violacea* attain the same conclusion that self-compatibility should be considered dominant over self-incompatibility, though there is a considerable discrepancy among F_2 segregation ratios in the results of these investigators. In the present author's case, however, self-compatible plants

were produced quite usually as the descendants of selfed self-incompatible plants. This fact is quite clear and, of course, should not be considered due to pseudo-self-fertility. The same phenomenon was observed also by CORRENS (1913) in *Cardamine pratensis,* by HERIBERT-NILSSON (1916) in rye, and by STOUT (1916, 1917, 1918, 1920, 1927) in several species of *Lilium* and *Linaria* and also in *Cichorium Intybus, Brassica pekinensis* and *B. chinensis.* Furthermore, STOUT (1927), after making several years' continued selection of self-compatible plants, says that he was neither able to secure any appreciable increase in the relative number of self-compatible plants nor to isolate any strain that continues to be self-compatible. From these facts, we are led to the question of the dominancy of self-compatibility. In order to solve this question, STOUT (1927) assumes a mutation such as a change from S_1S_2 to S_1S_0 occurring with decided lack of stability, S_0 having not any oppositional action and consequently S_1S_0 being self-compatible.

Recently, EAST and YARNELL (1929) have reported that they have isolated from material of the forms known as *Nicotiana alata grandiflora* and *N. Sanderae* fifteen allelomorphs for self-incompatibility, named S_1, S_2.... S_{15}, which all behave as allelomorphs to each other. Besides these, they have also assumed that the factor S_f, another S allelomorph coming from *N. Langsdorfii,* produces pollen tubes which grow so rapidly that no combination of inhibiting factors will cause self-incompatibility. Moreover, they presume the possibility that subsidiary factors at another locus than the S have effects upon the rapidity of pollen-tube growth in plants characterized by particular S factors, and believe that in a plant S_1S_2WW the pollen tube may grow faster after an incompatible mating than it will be in a plant S_1S_2ww.

Here still remains the problem of cross-incompatibility in the matings between different self-compatible plants and between self-compatible and self-incompatible ones. The crossing between the two self-compatible plants Nos. 2 and 8 in 1925 was incompatible reciprocally; the crossing between the self-compatible plant No. 15 and each of the three self-incompatible members of the class B in 1928 and that between the self-compatible plant No. 11–3 and each of the four self-incompatible members of the class A of the family No. 11 in 1929 were incompatible when the self-incompatible plants were used as females; and the crossing between the self-compatible plant No. 11–3 and the self-incompatible plant No. 11–5 in the same year was incompatible when the self-incompatible plant was used as male. No such phenomena have been reported before so far as the author is aware, and these seem impossible to be explained by any of the hypotheses heretofore propounded.

5) Interpretation of the Results

a) *A Hypothesis*

A new genetic hypothesis will be proposed here, in which two allelomorphic series acting just in contradictory manner to each other, the one oppositional and the other sympathetic, are assumed.

1. Triple allelomorphs $\mathbf{S}_1$, $\mathbf{S}_2$ and $\mathbf{S}_3$ of the oppositional character: Each of them, as there is no dominant action among them, exerts its respective influence on the style of pistil, so as to let it inhibit intensely the tube penetration of the pollen possessing the same factor. For instance, an $\mathbf{S}_1\mathbf{S}_2$ style allows only the $\mathbf{S}_3$ pollen tube to penetrate within it, but not the $\mathbf{S}_1$ and $\mathbf{S}_2$ pollen tubes.

2. A pair of allelomorphs $\mathbf{T}_1$ and $\mathbf{T}_2$ of the sympathetic character: As there is no dominant action between them, each exerts also its influence on the style, but just in contrary manner as the **S** series, because it accelerates the tube penetration of the pollen possessing the same factor. For instance, a $\mathbf{T}_1\mathbf{T}_1$ style accelerates only the penetration of the $\mathbf{T}_1$ pollen tube, but not that of the $\mathbf{T}_2$ pollen tube.

3. In regard to the interrelation between the **S** series and the **T** series, it is further assumed that the **S** series is epistatic to the **T** series, but the **T** in double dose is more active than the **S** in simple dose. For instance, in an $\mathbf{S}_1\mathbf{S}_1\mathbf{T}_1\mathbf{T}_1$ or $\mathbf{S}_1\mathbf{S}_1\mathbf{T}_2\mathbf{T}_2$ style $\mathbf{S}_1$ is active, in an $\mathbf{S}_1\mathbf{S}_2\mathbf{T}_1\mathbf{T}_2$ style both $\mathbf{S}_1$ and $\mathbf{S}_2$ are active, and in an $\mathbf{S}_1\mathbf{S}_2\mathbf{T}_1\mathbf{T}_1$ or $\mathbf{S}_1\mathbf{S}_3\mathbf{T}_1\mathbf{T}_1$ style $\mathbf{T}_1$ is active.

The nature of the opposional factors is quite similar to that of those assumed by East and Mangelsdorf (1925, 1926). The factors assumed by them, however, are subsidiary to the fundamental fertility factor **F**, and they operate only when the fundamental factor is in the recessive condition, while the oppositional factors in the present author's case are independent of such fundamental factors. The sympathetic factors bear a resemblance to the pollen-tube factors described by Jones (1928), which control the growth of pollen tubes and give disturbed segregation ratios in crosses, inasmuch as both promote the union of the gametes having the same constitution. There is a decided dominancy, however, between the pair of the Jones' pollen-tube factors, instead of no dominancy in the sympathetic factors in our case. Results indicating the presence of the pollen-tube factors have been reported in several plants by comparatively many investigators, such as in maize by Correns (1902), Collins and Kempton (1911), East and Hayes (1911), Kempton (1919), Brink and MacGillivray (1924), Emerson (1924), Jones (1924), Brink (1925), Coulter (1925), Kiessel-

BACH and PETERSON (1926), MANGELSDORF (1926), MANGELDORF and JONES (1926) and BRINK and BURNHAM (1927); in *Oenothera* by RENNER (1919, 1921), HERIBERT-NILSSON (1920, 1923), DE VRIES (1924) and DAVIS (1926); in *Melandrium rubrum* GARCKE by CORRENS (1921); in *Rumex acetosa* L. by CORRENS (1922); in *Datura* by BUCHHOLZ and BLAKESLEE (1922) and SIRKS (1926); in peas by SIRKS (1923) and WELLENSIEK (1925); in rice by PARNELL (1921); and in barley by HALLQVIST (1923). Yet, the two types of factors giving selective action of the growth of pollen tubes, the incompatibility factors and the pollen-tube factors as they are called, have not been reported to occur in the same species, so far as the present author is aware. Our hypothesis may be said, in a sense, to assume the synchronism of both types of factors.

b) *The Results in Succession*

It may be supposed that the plants of the five classes A, B, C, D and E in 1925 have the genotypical constitution $\mathbf{S_1S_1T_1T_2}$, $\mathbf{S_2S_3T_1T_2}$, $\mathbf{S_1S_2T_1T_2}$, $\mathbf{S_1S_3T_1T_1}$ and $\mathbf{S_1S_3T_2T_2}$, respectively, all the plants of each class being of the same constitution. The expected results of pollinations between the classes are shown in Diagram 5, in which those of intraclass crossings are not given as they ought to be the same as those of the selfing of each plant belonging to the same class. From the diagram, we see that in about half of the matings pollen is partly effective and partly ineffective. These matings would apparently be compatible just as the other compatible ones, but not be semi-incompatible when the pollen is supplied abundantly, because selective pollen-tube growth would occur. Thus, in all matings, the expected result agrees precisely with the actual one summarized in Diagram 1.

The self-compatible plants Nos. 2 and 8, having the genetic constitutions $\mathbf{S_1S_3T_1T_1}$ and $\mathbf{S_1S_3T_2T_2}$ respectively, will segregate, when selfed, into 1 $\mathbf{S_1S_1T_1T_1}$:2 $\mathbf{S_1S_3T_1T_1}$:1 $\mathbf{S_3S_3T_1T_1}$ and 1 $\mathbf{S_1S_1T_2T_2}$:2 $\mathbf{S_1S_3T_2T_2}$:1 $\mathbf{S_3S_3T_2T_2}$, respectively. Among these descendants, $\mathbf{S_1S_3T_1T_1}$ and $\mathbf{S_1S_3T_2T_2}$ will be self-compatible whereas all the others are self-incompatible, so that each of the families will contain 1 self-compatible and 1 self-incompatible. The actual result is 13 and 10 in the progeny of No. 2, and 4 and 5 in those of No. 8, as shown in Table V. In both cases, deviations from the ratio 1:1 are slight and come within the limits of the probable errors.

The self-incompatible plants Nos. 5 and 10 (Class B) having the constitution $\mathbf{S_2S_3T_1T_2}$ are heterozygous in respect to both **S** and **T**, and will, when some seeds are obtained by selfing, undergo the dihybrid segregation. Among the segregates, when we take the basic number 16, 2 $\mathbf{S_2S_3T_1T_1}$ and

2 $S_2S_3T_2T_2$ or those which are heterozygous concerning S but homozygous concerning T will be self-compatible, whereas the remainder, 12 in number, will be all self-incompatible. In other words, the segregation 1 self-compatible : 3 self-incompatibles will be obtained. The same segregation may be expected phenotypically from another self-incompatible plant No. 6 (Class C) which possesses the constitution $S_1S_2T_1T_2$. Although, as seen in Table VI, both of the two descendants of No. 6 were equally self-incompatible, this might be due to a too small number of plants; and the fact that one of the two descendants of No. 5 was self-compatible is a strong evidence for the

Pollen / Styles	A	B				C				D		E	
	S_1T_1	S_1T_2	S_2T_1	S_2T_2	S_3T_1	S_3T_2	S_1T_1	S_1T_2	S_2T_1	S_2T_2	S_1T_1	S_3T_1	S_1T_2	S_3T_2
A = $S_1S_3T_1T_2$	−	−	+	+	+	+	−	−	+	+	−	+	−	+
	S		F				F				F		F	
B = $S_2S_3T_1T_2$	+	+	−	−	−	−	+	+	−	−	+	−	+	−
	F		*S*				F				F		F	
C = $S_1S_2T_1T_2$	−	−	−	−	+	+	−	−	−	−	−	+	−	+
	S		F				*S*				F		F	
D = $S_1S_3T_1T_1$	+	−	+	+	+	−	+	−	+	+	+	+	−	−
	F		F				F				*F*		S	
E = $S_1S_3T_2T_2$	−	+	+	+	−	+	−	+	+	+	−	−	+	+
	F		F				F				S		*F*	

Diagram 5. The expected results of self- and cross-pollinations in 1925.
+, the case where the pollen is effective; and −, ineffective.

expectation described above. From No. 10, no selfed seed was obtained, so that its progeny could not be tested.

All the other self-incompatible plants Nos. 1, 3, 4, 7 and 9 (Class A), having the constitution $S_1S_1T_1T_2$, will give only self-incompatible descendants by selfing, for each of them will segregate into 1 $S_1S_1T_1T_1$: 2 $S_1S_1T_1T_2$: 1 $S_1S_1T_2T_2$, all of which will be self-incompatible. In agreement with this expectation, as seen in Table VI, all the descendants of the plants belonging to Class A, seven in total, were self-incompatible without exception.

Among the descendants of the self-compatible plant No. 2, all the self-compatibles will be of the constitution $S_1S_3T_1T_1$, and the self-incompatibles will belong to the two classes, $S_1S_1T_1T_1$ and $S_3S_3T_1T_1$. Then, the crossing between any two of the self-compatible descendants will be always compatible; but the crossings between the self-incompatibles will be incompatible in some cases while compatible in the others, i.e., will be incompatible when

the matings are between individuals of the same genetic constitution but compatible between those of the different constitutions. Furthermore, the crossings between the self-compatible descendants and the self-incompatible ones will be always compatible. The actual result of Table VII proves to be in precise agreement with these expectations. It is apparent that, among the three self-incompatible descendants used for the crossings, No. 2–2 belongs to one class and Nos. 2–6 and 2–21 to the other.

Finally, No. 1–1 might have, as mentioned above, either one of the constitutions $S_1S_1T_1T_1$, $S_1S_1T_1T_2$ or $S_1S_1T_2T_2$, though it is unknown which one is really correct. It might be, however, of the constitution either $S_1S_1T_1T_1$ or $S_1S_1T_1T_2$ but not $S_1S_1T_2T_2$. If it were of $S_1S_1T_2T_2$, its crossing with the self-compatible descendants ($S_1S_3T_1T_1$) of No. 2, when it was used as male, would be incompatible on account of ineffective S_1T_2 pollen only, which was however not the case, as shown in Table VIII. On the other hand, if it was of $S_1S_1T_1T_1$, the crossing between it and the self-compatible descendants of No. 2 would be compatible in both directions; because when it was used as pollen plant effective S_1T_1 pollen only would be produced, and in the reciprocal crossing S_3T_1 pollen would be effective. Further, the crossing between it and the self-incompatible descendants ($S_3S_3T_1T_1$ and $S_1S_1T_1T_1$) of No. 2 would be compatible in both directions when the descendants of No. 2 were of $S_3S_3T_1T_1$, but incompatible in both directions when those were of $S_1S_1T_1T_1$. In like manner, if No. 1–1 was of the constitution $S_1S_1T_1T_2$, it would be compatible either with $S_1S_3T_1T_1$ or $S_3S_3T_1T_1$ in both directions, but incompatible with $S_1S_1T_1T_1$. In fact, No. 1–1 was compatible either with all the self-compatible descendants of No. 2 or with the self-incompatible descendants No. 2–2 in both directions, but was incompatible with the other self-incompatibles, Nos. 2–6 and 2–21. This fact gives also another evidence in respect to the fact that No. 2–2 belongs to the one and Nos. 2–6 and 2–21 to the other of the two classes which are expected about self-incompatible descendants of No. 2, and it proves that the former descendant is of the constitution $S_3S_3T_1T_1$ and the latter two of that $S_1S_1T_1T_1$.

Thus the hypothesis given here explains very satisfactorily either the result of the self- and cross-pollinations of the plants in 1925 or that obtained from the experiment made with their progenies in 1927.

c) *The Results in Toyodawase*

Let us assume the factor constitutions of the members of the six classes in 1928 as follows:

$$A = S_1S_2T_1T_2$$
$$B = S_2S_3T_1T_2$$
$$C = S_1S_3T_1T_2$$
$$D = S_1S_1T_1T_2$$
$$E = S_1S_2T_1T_1 \quad (S_1S_2T_2T_2)^{(1)}$$
$$F = S_2S_3T_1T_1 \quad (S_2S_3T_2T_2)$$

Then, from the self- and cross-pollinations of these plants the results may be expected as shown in Diagram 6. The actual results presented in Diagram 2 are in good accord with the expectation.

The plants belonging to Classes A, B and C are heterozygous in both **S** and **T** factors, and their descendants grown from selfed seeds should display the dihybrid segregation as follows:

$1\ S_aS_aT_1T_1$	$2\ S_aS_aT_1T_2$	$1\ S_aS_aT_2T_2$
$2\ S_aS_bT_1T_1$	$4\ S_aS_bT_1T_2$	$2\ S_aS_bT_2T_2$
$1\ S_bS_bT_1T_1$	$2\ S_bS_bT_1T_2$	$1\ S_bS_bT_2T_2$

Among these segragates, those which possess a formula heterozygous **S** and homozygous **T**, viz., $2\ S_aS_bT_1T_1$ and $2\ S_aS_bT_2T_2$, will be self-compatible, but all the others are self-incompatible; so that self-compatible and self-incompatible plants are in 1:3 ratio.

The plant of Class D which has the constitution $S_1S_1T_1T_2$ should give, when selfed, $1\ S_1S_1T_1T_1 : 2\ S_1S_1T_1T_2 : 1\ S_1S_1T_2T_2$. These descendants will be all self-incompatible.

The plants of Classes E and F are heterozygous in **S** and homozygous in **T**. The descendants grown from their selfed seeds should consist of $1\ S_aS_aT_1T_1 : 2\ S_aS_bT_1T_1 : 1\ S_bS_bT_1T_1$. Among these, $2\ S_aS_bT_1T_1$ will be self-compatible, but the others self-incompatible; so that in this case a 1:1 ratio should be obtained.

When these expectations are compared with the actual results as shown in Table XXVII, it will be seen that the agreement is good in all cases.

Further, considerations will be made here regarding the results of cross-pollinations among the descendants in 1929. The genetic constitution of each plant of the family No. 11 may be considered as shown in Table XXVIII, where the result of the statistical study does not show any irrationality. Then the actual results of crossing presented before in Diagram 3 will be explained quite reasonably, as shown in Diagram 7.

(1) Though Classes E and F may be considered to have the factor constitutions shown within the parentheses, respectively, no different results will be obtained so far as the self- and cross-pollinations in the present experiments are concerned.

Pollen / Styles	A S_1T_1 S_1T_2 S_2T_1 S_2T_2	B S_2T_1 S_2T_2 S_3T_1 S_3T_2	C S_1T_1 S_1T_2 S_3T_1 S_3T_2	D S_1T_1 S_1T_2	E S_1T_1 S_2T_1	F S_2T_1 S_3T_1
$A = S_1S_2T_1T_2$	− − − − *S*	− − + + F	− − + + F	− − S	− − S	− + F
$B = S_2S_3T_1T_2$	+ + − − F	− − − − *S*	+ + − − F	+ + F	+ − F	− − S
$C = S_1S_3T_1T_2$	− − + + F	+ + − − F	− − − − *S*	− − S	− + F	+ − F
$D = S_1S_1T_1T_2$	− − + + F	+ + + + F	− − + + F	− − *S*	− + F	+ + F
$E = S_1S_2T_1T_1$	+ − + − F	+ − + + F	+ − + + F	+ − F	+ + *F*	+ + F
$F = S_2S_3T_1T_1$	+ + + − F	+ − + − F	+ + + − F	+ + F	+ + F	+ + *F*

Diagram 6. The expected results of self- and cross-pollinations in 1928.
Notations are the same as in Diagram 5.

Pollen / Styles	A				B	C			D	
	S_1T_1	S_1T_2	S_2T_1	S_2T_2	S_2T_2	S_1T_1	S_1T_1	S_1T_2	S_1T_1	S_2T_1
$A=S_1S_2T_1T_2$	−	−	−	−	−	−	−	−	−	−
		S			S	S	S		S	
$B=S_2S_2T_2T_2$	+	+	−	−	−	+	+	+	+	−
		F			*S*	F	F		F	
$C=S_1S_1T_1T_1$	−	−	+	+	+	−	−	−	−	+
		F			F	*S*	S		F	
$C=S_1S_1T_1T_2$	−	−	+	+	+	−	−	−	−	+
		F			F	S	*S*		F	
$D=S_1S_2T_1T_1$	+	−	+	−	−	+	+	−	+	+
		F			S	F	F		*F*	

Diagram 7. The expected results of the self- and cross-pollinations in the family No. 11 in 1929. Notations are the same as in Diagram 5.

In the same way, each member of the family No. 15 may be considered to be constituted as shown in Table XXIX, and the results presented before in Diagram 4 will be easily understood as given in Diagram 8.

Pollen / Styles	A	B	C	
	S_2T_1	S_3T_1	S_2T_1	S_3T_1
$A=S_2S_2T_1T_1$	−	+	−	+
	S	F	F	
$B=S_3S_3T_1T_1$	+	−	+	−
	F	*S*	F	
$C=S_2S_3T_1T_1$	+	+	+	+
	F	F	*F*	

Diagram 8. The expected results of the self- and cross-pollinations in the family No. 15 in 1929. Notations are the same as in Diagram 5.

The family No. 18 should be composed of 1 $S_1S_1T_1T_1$: 2 $S_1S_1T_1T_2$: 1 $S_1S_1T_2T_2$ as already mentioned, and the crossings among these plants should be incompatible always, all of them are besides self-incompatible, as shown in Diagram 9. This expectation was actually realized as already seen in Table XXVI.

Here remains a problem of the difference of the grades of fertility in both so-called "compatible" and "incompatible" pollinations. It seems that this problem has been treated of before rarely. In this connection, TERAO and U (1929) give their results of study as follows: They report that,

in *Petunia violacea,* various grades of self-fertility are found, which may be classified into six, and that, in a certain strain of the species, plants were regularly obtained that were semi-incompatible not only in selfing but also in crossing with individuals of certain self-incompatible classes. The results in 1929 of the present studies have shown that the variation

Females \ Males	$S_1S_1T_1T_1$ S_1T_1	$S_1S_1T_1T_2$ S_1T_1 S_1T_2	$S_1S_1T_2T_2$ S_1T_2
$S_1S_1T_1T_1$	— / *S*	— — / S	— / S
$S_1S_1T_1T_2$	— / S	— — / *S*	— / S
$S_1S_1T_2T_2$	— / S	— — / S	— / *S*

Diagram 9. The expected results of the self- and cross-pollinations in the family No. 18 in 1929. Notations are the same as in Diagram 5.

in the relative fertility of the compatible cross-pollinations was wider than that of the compatible self-pollinations. To examine the nature of these variations, the distribution of the normal curve has been calculated from the total result of all selfings and crossings in that year.(1) The calculated results are shown and compared with the actual ones in Tables XXX and XXXI.

It is seen that the variation in the relative fertility of the compatible self-pollinations gives a pretty good agreement with the distribution of the normal curve, although the value of deviation divided by the probable error in the last line is exceptionally somewhat large. Consequently, it may be said that this variation may be attributed merely to a chance fluctuation. The variation of the compatible cross-pollinations, however, shows a rather worse agreement with the distribution of the normal curve, and the result proves that the deviations are significant.

Moreover, the crossing result of the family No. 18, in which all the matings have been put into the "incompatible" class, shows a bimodal variation in the grade of fertility, as given in Table XXVI. Accordingly, it is difficult to regard the variation as to be due merely to a chance fluctation.

(1) The distribution of the normal curve has been calculated by the formula: $y_x = \frac{n}{\sigma\sqrt{2\pi}} \cdot \frac{1}{e^{x^2/2\sigma^2}}$, where y = the frequency of the normal curve, x = the difference of any class value from the median, σ = the standard deviation, and n = the total number of the variates.

These facts indicate that the factors investigated in the preceding pages function in more or less different intensities, or that, besides the factors, one or more factors of minute values are involved; so that, in both of the "compatible" and "incompatible" groups, there will be different grades of fertility due not only to physiological causes, but also to genetic factors.

6) General Consideration of the Genetic Behavior

If we will admit the hypothesis above described, we can expect the existence of four groups of cabbage plants in respect to their factorial constitutions and self-breeding habits, as follows: (Letters S and F within parentheses represent "self-incompatible" and "self-compatible", respectively.)

1. Homozygous plants:

$S_1S_1T_1T_1$ (S)
$S_1S_1T_2T_2$ (S)
$S_2S_2T_1T_1$ (S)
$S_2S_2T_2T_2$ (S)
$S_3S_3T_1T_1$ (S)
$S_3S_3T_2T_2$ (S)
} will breed true ..... = (S)

2. S-homo-, T-heterozygous plants:

$S_1S_1T_1T_2$ (S)
$S_2S_2T_1T_2$ (S)
$S_3S_3T_1T_2$ (S)
} will segregate into .....
1 $S_\alpha S_\alpha T_1T_1$ (S)
2 $S_\alpha S_\alpha T_1T_2$ (S)
1 $S_\alpha S_\alpha T_2T_2$ (S)
} = (S)

3. S- and T-heterozygous plants:

$S_1S_2T_1T_2$ (S)
$S_1S_3T_1T_2$ (S)
$S_2S_3T_1T_2$ (S)
} will segragate into .....
1 $S_\alpha S_\alpha T_1T_1$ (S)
2 $S_\alpha S_\alpha T_1T_2$ (S)
1 $S_\alpha S_\alpha T_2T_2$ (S)
2 $S_\alpha S_b T_1T_1$ (F)
4 $S_\alpha S_b T_1T_2$ (S)
2 $S_\alpha S_b T_2T_2$ (F)
1 $S_b S_b T_1T_1$ (S)
2 $S_b S_b T_1T_2$ (S)
1 $S_b S_b T_2T_2$ (S)
} =1 (F) : 3 (S)

4. S-hetero-, T-homozygous plants:

$S_1S_2T_1T_1$ (F)
$S_1S_2T_2T_2$ (F)
$S_1S_3T_1T_1$ (F)
$S_1S_3T_2T_2$ (F)
$S_2S_3T_1T_1$ (F)
$S_2S_3T_2T_2$ (F)
} will segregate into .....
1 $S_\alpha S_\alpha T_\alpha T_\alpha$ (S)
2 $S_\alpha S_b T_\alpha T_\alpha$ (F)
1 $S_b S_b T_\alpha T_\alpha$ (S)
} =1 (F) : 1 (S)

In regard to the self-breeding habits, it is here seen (1) that there are two kinds of self-incompatible plants, one which gives only self-incompatible offspring and the other which segregates into self-compatible and self-incompatible types in a ratio 1:3, and (2) that self-compatible plants always segregate into the two types in a ratio 1:1, but never breed true to the self-compatible type.

Taking these breeding habits into consideration, it is interesting to recall here that certain self-compatible individuals were obtained as the descendants of self-incompatible plants by CORRENS (1913) in *Cardamine pratensis*, by HERIBERT-NILSSON (1916) in rye, and by STOUT (1916, 1917, 1922, 1927) in several species of *Lilium, Cichorium, Brassica* and *Linaria*. It is also to be noticed that STOUT (1927), by means of continued selection of self-compatible plants of several species, was neither able to isolate any strain that continues to be self-compatible nor to obtain any appreciable increase in the relative number of self-compatible plants. These phenomena have not yet been explained very clearly, but this occurrence is quite natural, if the similar hypothesis as the present author's one is admitted.

Some considerations will be made below about the possible cases of cross-matings.

1. Matings between different self-compatible plants.—The possible result of the crossings among different self-compatible plants are given in Diagram 10.

The mating between two self-compatible plants which possess the same factor constitution is of course compatible just as in their selfing. When both members of a self-compatible couple are of different heterozygous constitution concerning **S**, such as S_1S_2 and S_1S_3 for instance, the mating will be cross-compatible in both directions whether the constitution in respect to **T** is similar or not. The possible matings of this type are of 12 sorts as follows:

between $S_1S_2T_1T_1(F)$ or $S_1S_2T_2T_2(F)$ and $S_1S_3T_1T_1(F)$ or $S_1S_3T_2T_2(F)$
between „ „ and $S_2S_3T_1T_1(F)$ or $S_2S_3T_2T_2(F)$
between $S_1S_3T_1T_1(F)$ or $S_1S_3T_2T_2(F)$ and „ „

Incompatibility in the crossing between different self-compatible plants will be observed only in such a mating that both members of a couple are of the same heterozygous constitution regarding **S** but of the different homozygous one regarding **T**. The following 3 sorts of matings are of this type:

between $S_1S_2T_1T_1(F)$ and $S_1S_2T_2T_2(F)$
between $S_1S_3T_1T_1(F)$ and $S_1S_3T_2T_2(F)$
between $S_2S_3T_1T_1(F)$ and $S_2S_3T_2T_2(F)$

The matings of this kind will be obtained among the self-compatible plants of different sources or among the self-compatible descendants of a self-incompatible plant, but never among the offspring from one self-compatible plant, as might be understood from the self-breeding habits described above.

2. Matings between different self-incompatible plants.—When both members of a self-incompatible couple possess **S** of different kinds, that is, for instance, when one bears S_1 but lacks S_2 while the other bears S_2 but

Males / Females	$S_1S_2T_1T_1$ (S_1T_1 S_2T_1)	$S_1S_2T_2T_2$ (S_1T_2 S_2T_2)	$S_1S_3T_1T_1$ (S_1T_1 S_3T_1)	$S_1S_3T_2T_2$ (S_1T_2 S_3T_2)	$S_2S_3T_1T_1$ (S_2T_1 S_3T_1)	$S_2S_3T_2T_2$ (S_2T_2 S_3T_2)
$S_1S_2T_1T_1$	+ + *F*	− − S	+ + F	− + F	+ + F	− + F
$S_1S_2T_2T_2$	− − S	+ + *F*	− + F	+ + F	− + F	+ + F
$S_1S_3T_1T_1$	+ + F	− + F	+ + *F*	− − S	+ + F	+ − F
$S_1S_3T_2T_2$	− + F	+ + F	− − S	+ + *F*	+ − F	+ + F
$S_2S_3T_1T_1$	+ + F	+ − F	+ + F	+ − F	+ + *F*	− − S
$S_2S_3T_2T_2$	+ − F	+ + F	+ − F	+ + F	− − S	+ + *F*

Diagram 10. The possible result of cross-pollinations between different self-compatible plants. Notations are the same as in Diagram 5.

lacks S_1, the mating will be cross-compatible in both directions, no matter how the constitution regarding **T** may be. The 39 sorts of matings shown by the lines in Diagram 11 are of this type.

The mating between the two self-incompatible plants which are of the same factor constitution is of course cross-incompatible in both directions just as in their selfing. When both plants of a couple are of the same homozygous constitution concerning **S**, the mating will be also cross-incompatible in both directions whether the constitution regarding **T** is similar or not. The possible matings of this type are 9 sorts as follows:

between any two of $S_1S_1T_1T_1$(S), $S_1S_1T_1T_2$(S) and $S_1S_1T_2T_2$(S)
between any two of $S_2S_2T_1T_1$(S), $S_2S_2T_1T_2$(S) and $S_2S_2T_2T_2$(S)
between any two of $S_3S_3T_1T_1$(S), $S_3S_3T_1T_2$(S) and $S_3S_3T_2T_2$(S)

When one member of a self-incompatible couple is homozygously constituted in respect to **S** and the other heterozygously in respect to it, and

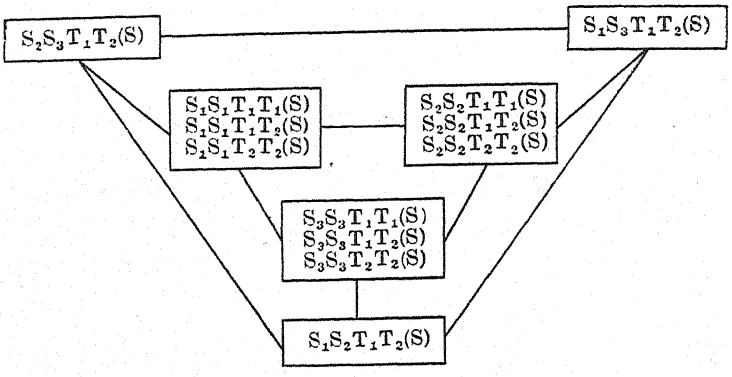


Diagram 11. The matings between different self-incompatible plants which will be cross-compatible in both directions.

besides one factor is common to both, i. e., for instance, when one is of the constitution S_1S_1 and the other S_1S_2, the mating will be cross-compatible if the former is used as female but incompatible in the reciprocal crossing whether the constitution regarding **T** is similar or not. The possible matings of this type are 18 sorts as shown in Diagram 12.

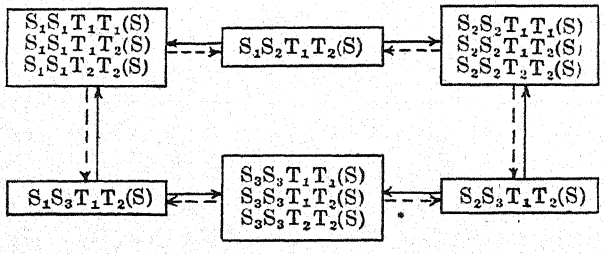


Diagram 12. The matings between different self-incompatible plants which will be cross-compatible in one direction but incompatible in the reciprocal crossing. The compatible direction is shown by the solid line, and the incompatible by the broken line.

3. Matings between self-compatible plants and self-incompatible ones. —When a self-incompatible plant is homozygous about **S** of any kind and also homozygous about **T** of the same kind as in a self-compatible plant, or when the former bears **S** of a kind different from that in the latter, the mating between the two plants will be cross-compatible in both directions. The 36 sorts of matings given in Diagram 13 belong to this type.

When a self-incompatible plant is heterozygous regarding **S** as in a self-compatible one, the crossing between the two plants will give the same

results as in the selfing of either one used as female. In other words, the mating will be cross-compatible when the self-compatible plant is used as

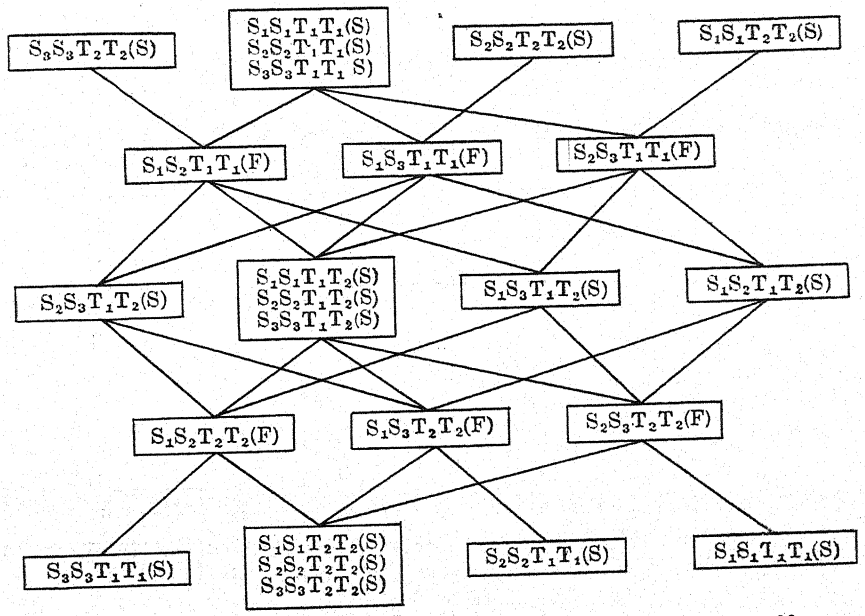


Diagram 13. The matings between self-compatible plants and self-incompatible ones which will be cross-compatible in both directions.

female, while it will be cross-incompatible when the self-incompatible one is used as female, as will be understood from Diagram 14. The matings of this type are 6 sorts as follows:

Males / Females	$S_1S_2T_1T_1$(F) S_1T_1 S_2T_1	$S_1S_2T_2T_2$(F) S_1T_2 S_2T_2	$S_1S_2T_1T_2$(S) S_1T_1 S_1T_2 S_2T_1 S_2T_2
$S_1S_2T_1T_1$(F)			+ − + − F
$S_1S_2T_2T_2$(F)			− + − + F
$S_1S_2T_1T_2$(S)	− − S	− − S	

Diagram 14. Examples of the cross matings between self-compatible plants and self-incompatible ones which will be compatible when the former are used as females but incompatible in the reciprocal crossings. F within the parentheses indicates "self-compatible", and S "self-incompatible". The other notations are the same as in Diagram 5.

between $S_1S_2T_1T_1$(F) or $S_1S_2T_2T_2$(F) and $S_1S_2T_1T_2$(S)
between $S_1S_3T_1T_1$(F) or $S_1S_3T_2T_2$(F) and $S_1S_3T_1T_2$(S)
between $S_2S_3T_1T_1$(F) or $S_2S_3T_2T_2$(F) and $S_2S_3T_1T_2$(S)

When a self-incompatible plant is homozygous regarding **S** which is of the same kind as one of the **S** pair of a self-compatible one, and yet is homozygous in respect to **T** of a kind different from that of the latter, the mating will, just contrary to the preceding case, give the same result

Males / Females	$S_1S_2T_1T_1$(F) ($\overbrace{S_1T_1\ S_2T_1}$)	$S_1S_1T_2T_2$(S) S_1T_2	$S_2S_2T_2T_2$(S) S_2T_2
$S_1S_2T_1T_1$(F)		$\overset{-}{S}$	$\overset{-}{S}$
$S_1S_1T_2T_2$(S)	$-\ \overset{+}{F}$		
$S_2S_2T_2T_2$(S)	$+\ \overset{-}{F}$		

Diagram 15. Examples of the cross matings between self-compatible plants and self-incompatible ones which will be compatible when the latter are used as females but incompatible in the reciprocal crossings. Notations are the same as in Diagram 14.

as in the selfing of either one which is used as male. This condition will be easily understood from Diagram 15. The possible matings of this type are of 12 sorts as follows:

between $S_1S_2T_1T_1$(F) and $S_1S_1T_2T_2$(S) or $S_2S_2T_2T_2$(S)
between $S_1S_2T_2T_2$(F) and $S_1S_1T_1T_1$(S) or $S_2S_2T_1T_1$(S)
between $S_1S_3T_1T_1$(F) and $S_1S_1T_2T_2$(S) or $S_3S_3T_2T_2$(S)
between $S_1S_3T_2T_2$(F) and $S_1S_1T_1T_1$(S) or $S_3S_3T_1T_1$(S)
between $S_2S_3T_1T_1$(F) and $S_2S_2T_2T_2$(S) or $S_3S_3T_2T_2$(S)
between $S_2S_3T_2T_2$(F) and $S_2S_2T_1T_1$(S) or $S_3S_3T_1T_1$(S)

In summarizing, the total number of the possible sorts of the matings between the plants genotypically different from each other will be $\frac{18^2-18}{2}$ $=153$, where 18 indicates the possible sorts of genotypes, and the matings will be grouped as follows:

1. Matings between different self-compatible plants:
   a) compatible in both directions, 12 sorts;
   b) incompatible in both directions, 3 sorts.
2. Matings between different self-incompatible plants:
   a) compatible in both directions, 39 sorts;

b) incompatible in both directions, 9 sorts;
c) compatible in one direction but incompatible in the other direction, 18 sorts.

3. Matings between self-compatible plants and self-incompatible ones:
a) compatible in both directions, 54 sorts;
b) compatible in one direction but incompatible in the other direction, showing the same result as in the selfing of those used as females, 6 sorts;
c) compatible in one direction but incompatible in the other direction, showing the same result as in the selfing of those used as males, 12 sorts.

II. Physiological Studies

1) Material and Methods

In the spring of 1929, three flowering plants, viz. A, B and C, of the variety Toyodawase were chosen for the purpose of the experiments which will be reported below. A special attention was paid for selecting those plants which have a large number of flowering branches, because the experiments planned require the presence of many inflorescences of possibly uniform vigor on each plant.

The object of experiments was double, one refering to the rate of pollen-tube growth and the other to the fertility in the flowers of different ages.

First experiments: A number of inflorescences which were uniform in vigor were chosen on Plant A, and 15 or 20 flower-buds situated at the middle part of each of the selected inflorescences were castrated one or two days before their opening, the remaining flowers and flower-buds being cut off. Each inflorescence was covered with a paraffined paper bag when the first castration was made. At 6 a.m., every day after it, the inflorescences were examined and the opened flowers were pollinated with pollen from fresh flowers of other bagged ones of the same plant (self-pollination) or of Plant B (cross-pollination). After such pollination a small tag indicating the time of pollination was put on each flower. Then, stigmas were cut off after pollination 6, 12, 24, 36, 48, 72 and 96 hours, respectively.

In an experiment of this kind, it may be desirable to remove not only stigmas but styles. However, the small upper part forming style of the pistil in the cabbage is somewhat different from the style in many other plants, as noticed by Pomel (1883), Hayek (1911), Schulz (1919) and others, and will bear very frequently one seed in itself. The upper part

may be, in certain other cruciferous plants such as *Raphanus*, the main part containing ovules. This peculiarity of the style becomes more comprehensible when the fact is considered that in radish-cabbage hybrids both upper and lower parts of pistil are equally developed and can bear seeds equally, as shown by KARPECHENKO (1924, 1927, 1928) and also by the present author (1927). Consequently, in the present experiment the stigma only was removed.

Second experiment: This experiment involves self-pollinations of Plants A, B and C, and cross-pollinations of Plant A with pollen from Plant B. A number of inflorescences on each of the three plants were selected, and all flowers and flower-buds were discarded except 15 or 20 situated at the middle part of each. In a part of the inflorescences all selected flower-buds were castrated at a time and pollinated at once, which may be called "premature pollination" or "bud-pollination." The inflorescence thus pollinated was covered with a bag bearing the date of operation. Thereafter, the inflorescence was examined every day, and to each opened flower a small tag was put, on which the number of days from pollination till blooming was noted. Thus, five lots, viz., those pollinated 1, 2, 3, 4 and 5 days before blooming, were experimented. In the rest of the inflorescences, just as in the preceding experiment, the flower-buds were castrated one or two days before blooming, and the inflorescence was bagged when the first castration was practised. Then, at 10 a.m. every day after the first castration, the inflorescences were examined and to each flower which had bloomed a small tag with the date was put. The bloomed flowers were pollinated at 10 a.m. of the 1st, 2nd, 3rd or 4th day of blooming.

In both experiments, the author planned to obtain the fertility results as accurate as possible. Such results could be secured only by counting separately ovules which had grown to seeds as well as those which had failed to become seeds. This counting of ovules was done very satisfactorily on premature fruits in the stage between about two weeks after pollination and just before discoloration of the fruits. The premature fruit was splitted carefully along the midrib with a needle, and both kinds of ovules at one side of the fruit were counted separately by help of a magnifying lens, and then the similar process was repeated on the other side.

2) Natural Fertility of the Plants Used for the Experiments

Fertility under the natural condition may be considered in general to show the highest fertility of respective plants, though some ovules may fail to become seeds. It is possible also that the percentage of ovules which grow

to seeds under the natural condition is comparatively higher in some plants than in others. If such is the case, it may be sometimes necessary to compare the fertility through a certain mode of pollination on a plant with that under the natural condition of the same plant. From these points of view, the natural fertility of each plant used for the experiments was observed, and the results are given in Table XXXII.

The results show that the three plants under experimentation bear about 40 to 60 percent fertile ovules in average, respectively, indicating that the grades of fertility under the natural condition may be dissimilar pretty considerably according to different plants. The percentages of fertile ovules in different fruits on a single plant also show a fairly wide variation, but the coefficients of variability are quite uniform, coming around 20 percent in all the three plants under experimentation.

3) Fertility Result of the Flowers of which Stigmas were Removed at Different Hours after Pollination

Table XXXIII shows the results of the self- or cross-pollinated flowers of which stigmas were removed at different hours after pollination or left unremoved, and besides the comparison between the average fertility in the control plot in which the stigmas were left unremoved and that in each of the other plots. The results are graphically represented in Fig. 1.

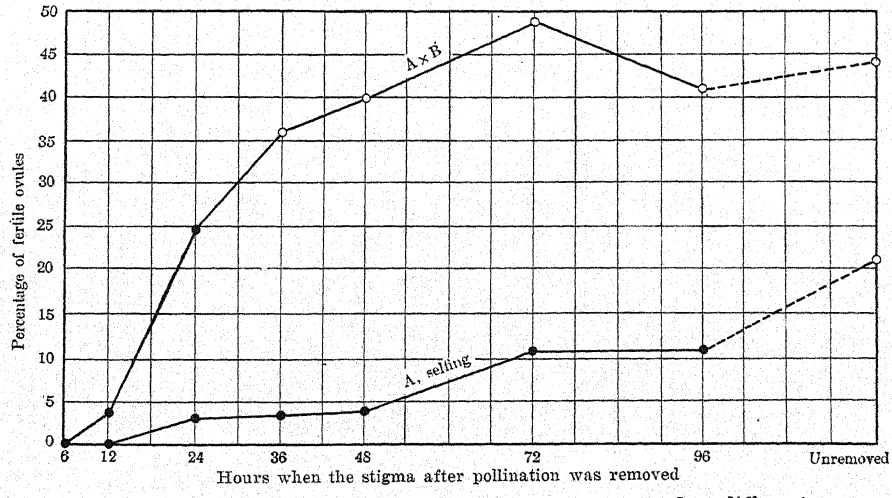


Fig. 1. Fertility of flowers of which stigmas were removed at different hours after pollination. The black spot and the circle represent, respectively, the fertility of which difference from the "Unremoved" is more than or less than three times the size of the probable error.

A few words may be given here in regard to the fertility nature of Plant A used for this experiment. This plant gave 21 percent fertile ovules by self-pollination. This fertility is rather high, nevertheless it is apparent that this plant is not truly self-compatible but self-incompatible showing comparatively higher fertility, because it is far lower than that either by the natural pollination or the cross-pollination with pollen from Plant B, as shown in Table XXXIV. The fact that this plant was self-incompatible and yet shows comparatively higher fertility by self-pollination was invaluable for our experimentation, because, if it were very lowly fertile by selfing, though not quite sterile, the grades of fertility in all the different plots of self-pollination would closely resemble each other, and consequently, we could hardly attain the very object of our experiment.

The mating between Plant A as female and Plant B as male is apparently a cross-compatible one, since the cross-pollination of this mating shows a fertility closely resembling that in the case of natural pollination of the female plant. The difference in fertility between the cross- and the natural pollinations is slight and comes within the limit of the probable error, as shown in Table XXXIV.

The result of self-pollination of Plant A indicates that the fertility of the flowers of which stigmas were removed 96 hours after pollination is 10.20 percent lower than that of the flowers of which stigmas were left unremoved, the difference being 4.3 times the size of the probable error. In other words, in the incompatible pollination, the fertility did not reach the maximum even 96 hours after pollination.

In the cross-pollination of the same plant with pollen from Plant B, the difference in fertility of each plot of 36, 48, 72, and 96 hours from the "unremoved" plot comes within three times the size of the probable error, so that they are insignificant. In other words, in the compatible cross-pollination, the fertility reaches almost the maximum as early as only 36 hours after pollination.

4) Fertility Result of the Flowers of Different Ages

Table XXXV shows the results of the self-pollinations of Plants A, B and C and the cross-pollinations of Plant A with pollen from Plant B made at different ages of flowers. The comparison between the fertility of the flowers on the first day of blooming and that of the flowers of other different ages is made also in that table. Fig. 2 shows the same results graphically.

It has been already mentioned that Plant A is ordinarily self-incompatible, and that the mating A×B is cross-compatible. Plants B and C are

both also self-incompatible without question, since their fertility in the self-pollination on the first day of blooming are 3.55 and 6.46 percent, respectively, and far lower than the fertility under the natural pollination of the

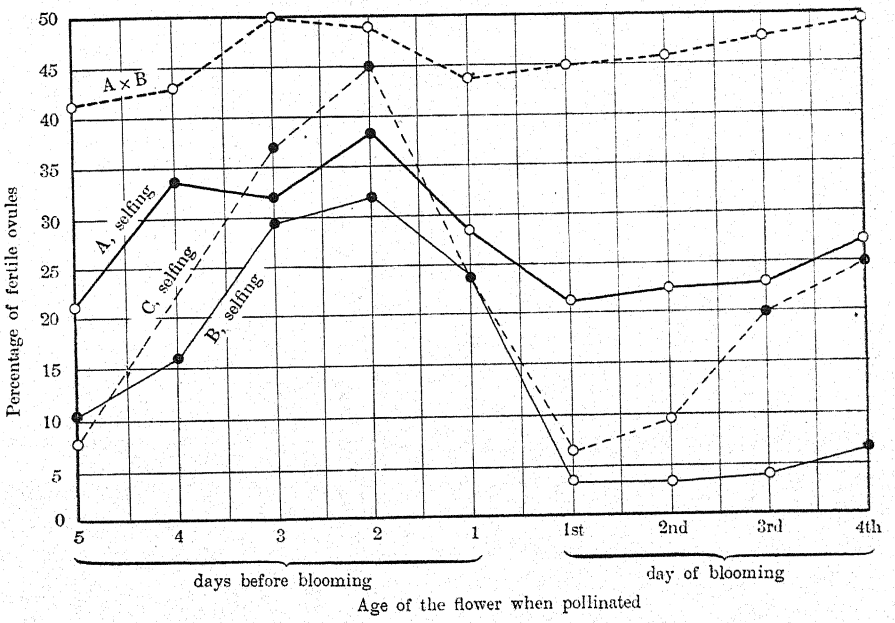


Fig. 2. Fertility of the flowers of different ages. The black spot and the circle represent, respectively, the fertility of which difference from the fertility of flowers on the first day of blooming is more than or less than three times the size of the probable error.

respective plants. The three plants are ordinarily all self-incompatible, but only the cross-mating A×B is compatible, so far as the matings in the experiment are concerned.

In all cases of the incompatible self-pollination, the bud-pollination increased fertility to a considerable degree, except when the buds pollinated were too young. The increased grade of fertility is highest in the pollination at two days before blooming; the fertility in this case is rather near that under the natural pollination, the former being about 80 percent of the latter, as shown in Table XXXVI.

Pollination of old flowers had a tendency also to increase the grade of fertility, though not so conspicuous as in bud-pollination. In the pollination on the fourth day on Plant B, and on the third day and the fourth day on Plant C, the increases in fertility over the pollination on the first day are

3.4, 6.7 and 5.6 times, respectively, the size of the respective probable errors, and they are naturally quite significant. The flowers in their fourth day of blooming had much withered petals which were prevented from falling by covering them with a bag; their stigmas had grown considerably. It is interesting to see that such old flowers gave higher fertility than the flowers in their prime.

In the compatible cross-pollination A×B, all the young and old flowers did not give any significant difference of fertility, as the difference of every plot from the plot of the first day comes within three times the size of the probable error.

5) Discussion

It is the problem of prime importance in the physiology of self- and cross-incompatibility whether it is brought about by the failure of pollen tubes to reach the egg or by the failure of zygote formation after the male nucleus has entered the embryo sac. Many evidences reported by certain investigators seem to show that the self- and cross-incompatibility in many plants is due to the slow rate or even the suspension of pollen-tube growth. It seems probable, on the other hand, as STOUT (1920) and FLORIN (1927) pointed out, that in certain cases abortion of embryos during early stages causes incompatibility. Very little evidence seems to be, however, present in such cases, so far as the author is aware.

JOST (1907) is perhaps the first investigator who reported the result of observation on the pollen-tube growth in the case of both compatible and incompatible pollinations. He made studies on *Lilium* and pointed out that in a self-incompatible plant the tubes from its own pollen are so limited in their development that fertilization does not occur, while in the case of cross-pollination the necessary length of pollen tube is easily attained.

In red clover, MARTIN (1913) observed that though in self-pollination the pollen germinates readily on the stigma, the tubes traverse the style much more slowly than in cross-pollination. WESTGATE and COE (1915), COE and MARTIN (1920), and WILLIAMS (1925) found that, when flowers of this plant were self-pollinated in the bud stage, a higher percentage of seeds was set than when they were selfed during blooming, and WILLIAMS says that this fact may be correlated with the slow growth of the pollen tube in selfed flowers.

In *Nicotiana,* EAST and PARK (1918) found that the pollen of a self-incompatible plant germinates on its own stigmas just as does the foreign pollen of another plant which is compatible with the former, and

the first increment of growth takes place at the same time. From that time on, the pollen tubes tend to grow steadily, but the rate of growth of the foreign pollen tubes is considerably accelerated. SMITH (1924, 1926) obtained, in both self-incompatible and cross-incompatible pollinations of *Nicotiana,* somewhat many seeds when unopened buds were pollinated while mature flowers set no seed, and concluded that the gametes are not incompatible and that fertilization occurs readily even in normally incompatible matings if male gametes can reach the egg. EAST and MANGELSDORF (1925, 1926, 1927) observed also the same phenomenon by pollinating young buds of *Nicotiana,* and concluded that self- and cross-incompatibility is due simply to a slow rate of pollen-tube growth and not to a true incompatibility between the gametes which will prevent zygote formation after the male nucleus has entered the micropyle.

KRAUS (1915) thinks that in self-incompatible apple varieties the union of the proper nuclei within the embryo sac generally takes place. NAMIKAWA (1923), examining the growth of pollen tubes in self-pollinated and exposed flowers of the Yellow Bellflower apple at different stages up to 72 hours, observed that the growth of pollen tubes in selfed flowers is not in any way abnormal or delayed. On the contrary, OSTERWALDER (1910) found that in self-pollinated apples the pollen tubes do not grow deep enough in the style, and KNIGHT (1918) expressed an opinion that an important factor which causes self-incompatibility in apples is the relatively slow rate of pollen-tube growth. Thus, in apples there are some results and opinions which are contradictory.

ASAMI's(1926) result of Japanese pears is in accord with OSTERWALDER's one. According to him, in self-pollinated flowers of the self-incompatible variety Chojuro, the pollen germinates on the stigmas and the tubes penetrate just as well as in flowers cross-pollinated with pollen from the variety Imamura-aki; but the pollen tubes in the former do not reach even the loculus after seven days while in the latter they reach the egg within a few days.

In both self- and cross-incompatible pollinations of plums and cherries, CRANE, (1925, 1927) observed that the pollen tubes are arrested in the stylar tissue and fail to reach the ovary, and that not only the ends of the pollen tubes swell up but in addition a slime sheath is formed around them.

The opinion that self- and cross-incompatibility is caused by the slow rate or suspension of pollen-tube growth is held also by certain other scientists who investigated various kinds of plants, such as CORRENS (1912) on *Cardamine,* COMPTON (1913) on *Reseda,* MOORE (1917) on *Tradescantia,* DORSEY

(1923) on apples, cherries and plums, and YASUDA (1928, 1929) on *Petunia*.

In the present experiments with the cabbage, in the case of incompatible self-pollination the grade of fertility in the flowers of which stigmas were removed as late as 96 hours after pollination was lower than in those of which stigmas were left unremoved, so that fertilization has taken place slowly and steadily; while in compatible cross-pollination the flowers of which stigmas were removed 36 hours after pollination did not show any appreciable difference in the grade of fertility from those left unoperated, so that fertilization in this case must have finished in very short time. This fact indicates apparently that under incompatible pollination the pollen tubes need a much longer time to reach the egg than under compatible pollination. In other words, incompatibility in the cabbage is caused by the slow rate of pollen-tube growth, just as in many other examples.

SASAOKA (1928) carried out a histological investigation on the growth of pollen tubes in cabbage flowers. He observed that, between self-pollinated flowers of the variety Toyodawase and cross-pollinated ones of the same variety with pollen from the variety Baby Head, no appreciable difference was observed in the course of the pollen-tube growth. One may say that this fact is inconsistent with the present author's result and indicates that self-incompatibility at issue is due to physiological repulsion between pollen and ovule nuclei or to embryo abortion. Before jumping to such a conclusion, however, it is necessary to make some consideration on the compatibility nature of the pollinations studied. SASAOKA gave the fertility result that seeds set per flower in the year of the histological study averaged 6.99 in self-pollinated Toyodawase and 4.57 in the same variety cross-pollinated with pollen from Baby Head, and added that the low fertility in the cross-pollination was due partly to the weak vegetative growth in that year and partly to pollination made too late in the season. What SASAOKA says about the cause of the low fertility in the cross-pollination may be perhaps quite right. We must take notice, however, that this fertility result is not derived from the very same individual plant as that from which material for the histological investigation was taken. Consequently, it might not be improbable that both the self- and cross-pollinations in the histological investigation were essentially the same as to the compatibility nature.

The second problem is whether the slow rate of pollen-tube growth in the case of incompatible pollination is brought about by a positive inhibiting action of the stylar tissue or by its lacking an accelerating action. EAST and MANGELSDORF (1926) say that "it is not certain whether similar genetic

formulae cause inhibited growth, or whether different genetic formulae cause accelerated growth.''

In the case that only one factor series is assumed as shown by many investigators, it is difficult, in the present author's opinion, to consider the slow rate of pollen-tube growth as due to the lacking of the accelerating action of the growth. If different genetic formulae cause the accelerated growth and similar genetic formulae do not affect the growth, either S_1 or S_2 pollen, for instance, may be effective on S_1S_2 pistil because S_1 pollen will be accelerated by S_2 factor of the pistil and S_2 pollen by S_1. Such is, however, not the case in fact.

In the genetic studies carried out by the present author, the presence of two factor series has been assumed, of which the one inhibits but the other accelerates the tube growth of the same pollen, respectively, as mentioned before. The physiology of these genetic factors may be explained very satisfactorily in the following manner: When the oppositional and sympathetic factors are present both in either simple or double dose, a substance which is secreted in the stylar tissue by the action of the oppositional factor and inhibits the tube growth of pollen predominates over that which is secreted by the sympathetic factor and accelerates the tube growth; but when the oppositional factor is present in simple dose and the sympathetic factor in double dose, the inhibiting substance is prevented in its action by the accelerating one.

At any rate, it may be concluded naturally that the slow rate of pollen-tube growth in the case of incompatible pollination is due positively to the presence of a substance which inhibits the growth in the stylar tissue, and the normal growth of pollen tubes in that of compatible pollination is a result of either the absence of such an inhibiting substance or the presence of an accelerating substance which is able to prevent the inhibiting action.

The third problem bears on the modification of the pollen-tube growth by the inhibiting action. It seems to be an unanimous opinion of the investigators who obtained the increased fertility by bud-pollination in incompatible matings, that the phenomenon of the increased fertility in such cases is due to the longer duration of time at disposal for the growth of pollen tubes as well as the shorter distance to be traversed. These opinions seem to me quite reasonable. Now, if the phenomenon were attributable solely to such causes, the grade of fertility of old flowers under incompatible pollination should decrease gradually.

The result of the author's experiment indicates on the contrary that under incompatible matings not only does the pollination of young buds

give increased fertility to a considerable degree, but also that of old flowers shows a gradual tendency to increase the fertility. In the pollination performed on the fourth day of blooming, no decrease of fertility was observed but the increase was quite conspicuous in two cases out of three, in spite of the fact that the flowers at this stage were so old that the petals would fall if they were not protected against wind by the bag. This fact indicates evidently the weakening of the inhibiting action of the pollen-tube growth in old flowers.

It may then be quite natural to consider that the increased fertility in bud-pollination is also due mainly to insufficient, if any, inhibiting action owing to immaturity of the style, and, in addition, to the longer time duration for the growth of pollen tubes as well as the shorter distance to be traversed.

The result of EAST and MANGELSDORF (1925, 1926) in *Nicotiana* seems to confirm this point. For instance, when they pollinated an $\mathbf{S_2S_3}$ plant in the very young bud stage with pollen from an $\mathbf{S_1S_3}$ plant, the resulting population showed that in this case an almost equal number of compatible ($\mathbf{S_1}$) and incompatible ($\mathbf{S_3}$) pollen tubes reached the ovary. From this fact they considered that "the growth curves of the two types did not have the opportunity to show extreme differentiation because of the relatively short distance traversed." This may be explained also by the present author's hypothesis that the incompatible pollen tubes grow in almost the same speed as do the compatible ones because of almost entire lacking of the inhibiting action owing to too young styles.

Furthermore, the increased fertility at the end of the flowering season which is called "end-season fertility" was observed by STOUT (1922, 1923) in *Lythrum,* and EAST (1923) and ANDERSON (1924) in *Nicotiana.* To explain this phenomenon, EAST and MANGELSDORF (1925, 1926) say that the individual flower remains longer time on the plant toward the end of the flowering season than at the beginning, so that the longer time is at the disposal for the growth of pollen tubes. On the other hand, YASUDA (1929), making an experiment with three self-incompatible plants of *Petunia violacea,* found that in each case the senile plant shows distinctively a higher grade of fertility than its sib which has been rejuvenated by cutting, when both are pollinated at the same time. STOUT (1922) observed that self-incompatibility in *Brassica pekinensis* and *B. chinensis* is strongest during the period of mid-bloom, and says that "self-incompatibility appears coincidentally with the climax of reproductive activity." When these results are taken into consideration, it is quite natural to conclude that the end-season fertility is also due to an insufficient inhibiting action caused by waning

senility rather than to the longer blooming time of the individual flower.

In conclusion, it is considered that the inhibiting substance which affects the growth of pollen tubes is secreted most abundantly when the pistil is in its full vigor and the secretion declines with the decline of vigor of the pistil. Such decline of vigor of the pistil may occur either when the flower blooms on a plant which is weakly, too young or old, or when the flower itself is too young as in the bud stage or too old.

Conclusion

1. Besides the known phenomena of the behavior of self- and cross-incompatibility, the author has observed certain new facts, such that a certain mating between two different self-compatible plants is incompatible reciprocally, and that, between self-compatible plants and self-incompatible ones, some matings are compatible only when the former are used as females while some others are compatible only when the latter are used as females. These facts are impossible to be explained by any of the hypotheses now existing.

2. Self-compatible plants were obtained quite usually as descendants of self-incompatible ones; the same phenomenon was reported before in certain species, but it might be said that no satisfactory interpretation had been given. Moreover, it was observed that self-compatible plants, when selfed, always segregated into both self-compatible and self-incompatible types. It is impossible also to explain this fact, by the hypothesis that self-compatibility is given by the factor **F** and self-incompatibility is characterized in its recessive condition.

3. All the results obtained by the author are explained very satisfactorily by a new genetic hypothesis that two contradictory allelomorphic series, one oppositional $\mathbf{S}_1$, $\mathbf{S}_2$ and $\mathbf{S}_3$ and the other sympathetic $\mathbf{T}_1$ and $\mathbf{T}_2$, affect the pollen-tube growth through the stylar tissue, and the **S** series is epistatic over the **T** series, but the **T** in double dose is more active than the **S** in simple dose.

4. Variations in the grade of fertility either in compatible crossings or in incompatible crossings are not only those due merely to chance fluctuations; and it is presumed that the allelomorphs described above function in more or less different intensities, or that, besides these, one or more factors of minute values are involved.

5. When selfed, some self-incompatible plants breed true to type while the other self-incompatible ones segregate into self-compatible and self-

incompatible types in a ratio 1:3, and self-compatible plants always segregate into the two types in a ratio 1:1 but never breed true to the self-compatible type.

6. Between different self-compatible plants, some matings are compatible reciprocally but the others are incompatible reciprocally. Between different self-incompatible plants, some matings are compatible reciprocally, some others are incompatible reciprocally, and the remainder are compatible in one direction but incompatible in the other. Between self-compatible plants and self-incompatible ones, some matings are compatible reciprocally, some others give the same result as in the selfing of those used as females, and the remainder give the same result as in the selfing of those used as males.

7. Self- and cross-incompatibility in the cabbage is caused by the slow rate of pollen-tube growth.

8. The slow rate of pollen-tube growth under incompatible pollination is due positively to the presence of a substance which inhibits the growth in the stylar tissue, and the normal growth of pollen tubes under compatible pollination is a result of either the absence of such an inhibiting substance or the presence of an accelerating substance which is able to prevent the inhibiting action.

9. For the production of the inhibiting substance the **S** allelomorphs and for that of the accelerating one the **T** allelomorphs are respectively responsible.

10. The inhibiting substance is produced most abundantly when the pistil is in its full vigor, and its production declines with the decline of vigor of the pistil. The pseudofertility in the case of bud-pollination of incompatible matings is very conspicuous in the cabbage. This is due mainly to insufficient inhibiting action owing to immaturity of the style, and also to the longer time duration at disposal for the growth of pollen tubes as well as the shorter distance to be traversed.

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Appendix

TABLE I. *The Result of Self- and Cross-pollinations in 1925*

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower
		Plant 1 (♀)			
1 (selfed)	*8*	*3*	*5*	*5*	*0.6*
2	6	6	0	157	26.2
3	7	0	7	0	0
4	6	1	5	1	0.2
5	5	5	0	81	16.2
6	5	5	0	124	24.8
7	5	1	4	2	0.4
8	7	7	0	112	16.0
9	6	1	5	6	1.0
10	5	5	0	97	19.4
		Plant 2 (♀)			
1	9	9	0	205	22.8
2 (selfed)	*7*	*7*	*0*	*159*	*22.7*
3	7	7	0	188	26.9
4	10	10	0	239	23.9
5	8	8	0	177	22.1
6	7	7	0	152	21.7
7	6	6	0	151	25.2
8	7	7	0	79	11.3
9	7	7	0	168	24.0
10	8	8	0	142	20.3
		Plant 3 (♀)			
1	9	0	9	0	0
2	10	10	0	224	22.4
3 (selfed)	*7*	*3*	*4*	*6*	*0.9*
4	9	2	7	17	1.9
5	8	8	0	170	21.2
6	9	9	0	186	20.7
7	9	7	2	35	3.9
8	9	9	0	167	18.6
9	8	3	5	5	0.6
10	9	9	0	235	26.1

TABLE I. (*Continued*)

Source of pollen (Plant No.)	Number of flowers, tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower
		Plant 4 (♀)			
1	8	5	3	15	1.9
2	6	6	0	111	18.5
3	6	1	5	1	0.2
4 (selfed)	*8*	*2*	*6*	*4*	*0.5*
5	8	8	0	126	15.8
6	6	6	0	207	34.5
7	6	4	2	19	2.7
8	7	7	0	141	20.1
9	7	1	6	1	0.1
10	7	7	0	136	19.4
		Plant 5 (♀)			
1	6	6	0	163	27.2
2	7	7	0	192	27.4
3	6	6	0	180	30.0
4	8	8	0	180	22.5
5 (selfed)	*8*	*2*	*6*	*3*	*0.4*
6	9	9	0	184	20.4
7	7	7	0	139	19.9
8	6	6	0	187	31.2
9	10	10	0	250	25.0
10	6	0	6	0	0
		Plant 6 (♀)			
1	6	0	6	0	0
2	7	7	0	163	23.3
3	8	0	8	0	0
4	10	1	9	1	0.1
5	7	7	0	193	27.6
6 (selfed)	*9*	*1*	*8*	*2*	*0.2*
7	7	2	5	3	0.4
8	9	9	0	219	24.3
9	7	2	5	6	0.9
10	8	8	0	223	27.9

TABLE I. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower
Plant 7 (♀)					
1	5	1	4	1	0.2
2	8	8	0	272	24.0
3	9	0	9	0	0
4	11	0	11	0	0
5	7	7	0	215	30.7
6	6	6	0	187	31.2
7 (selfed)	*6*	*0*	*6*	*0*	*0*
8	8	8	0	233	29.1
9	5	0	5	0	0
10	8	8	0	270	33.8
Plant 8 (♀)					
1	9	9	0	239	26.6
2	9	9	0	44	4.9
3	8	8	0	194	24.3
4	9	9	0	233	25.9
5	10	10	0	266	26.6
6	10	10	0	277	27.7
7	6	6	0	146	24.3
8 (selfed)	*8*	*8*	*0*	*172*	*21.5*
9	9	9	0	219	24.3
10	9	9	0	215	23.9
Plant 9 (♀)					
1	9	0	9	0	0
2	8	8	0	147	18.4
3	8	0	8	0	0
4	6	0	6	0	0
5	10	10	0	203	20.3
6	7	7	0	130	18.6
7	9	2	7	3	0.3
8	9	9	0	182	20.2
9 (selfed)	*8*	*3*	*5*	*3*	*0.4*
10	9	9	0	175	19.4

TABLE I. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower
		Plant 10 (♀)			
1	5	5	0	105	21.0
2	7	7	0	160	22.9
3	6	6	0	130	21.7
4	6	6	0	90	15.0
5	7	7	0	46	6.6
6	8	8	0	192	24.0
7	7	7	0	134	19.1
8	6	6	0	121	20.2
9	6	6	0	128	21.3
10 (selfed)	*9*	*0*	*9*	*0*	*0*

TABLE II. *The Average Number of Seeds per Flower in 1925*

Pistillate plants: Class	Plant No.	Pollen plants: A 1	A 3	A 4	A 7	A 9	B 5	B 10	C 6	D 2	E 8
A	1	*0.6*	0	0.2	0.4	1.0	16.2	19.4	24.8	26.2	16.0
	3	0	*0.9*	1.9	3.9	0.6	21.2	26.1	20.7	22.4	18.6
	4	0.5	0.2	*0.5*	2.7	0.1	15.8	19.4	34.5	18.5	20.1
	7	0.2	0	0	*0*	0	30.7	33.8	31.2	24.0	29.1
	9	0	0	0	0.3	*0.4*	20.3	19.4	18.6	18.4	20.2
B	5	27.2	30.0	22.5	19.9	25.0	*0.4*	0	20.4	27.4	31.2
	10	21.0	21.7	15.0	19.1	21.3	6.6	*0*	24.0	22.9	20.2
C	6	0	0	0.1	0.4	0.9	27.6	27.9	*0.2*	23.3	24.3
D	2	22.8	26.9	23.9	25.2	24.0	22.1	20.3	21.7	*22.7*	11.3
E	8	26.6	24.3	25.9	24.3	24.3	26.6	23.9	27.7	4.9	*21.5*

TABLE III. *Variations of Matings in the Average Number of Seeds per Flower in 1925*

	Number of seeds	Plant No. 1	3	4	7	9	5	10	6	2	8
Incompatible matings	0	1	1		4	3	1	1	2		
	0.1– 4.0	4	4	5	1	2	1		4		
	4.1– 8.0							1			1
	8.1–12.0									1	
Compatible matings	12.1–16.0	1		1				1			
	16.1–20.0	2	1	2		3	1	1			
	20.1–24.0		3	1	1	2	2	6	1	7	2
	24.1–28.0	2	1				3		3	2	7
	28.1–32.0				3		2				
	32.1–36.0			1	1						

TABLE IV. *The Result of Self-pollinations of the Progenies of Self-compatible Plants in 1927*

Mother-plant Class	Mother-plant No.	Plant No. of the progeny	Number of flowers: tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Fertile or sterile
D	2	1	3	3	0	60	20.0	F
		4	4	4	0	95	23.7	F
		8	5	5	0	91	18.2	F
		9	4	4	0	62	15.5	F
		11	5	5	0	92	18.4	F
		12	4	4	0	91	22.7	F
		13	4	4	0	75	18.7	F
		14	5	5	0	84	16.8	F
		17	4	4	0	68	17.0	F
		18	5	5	0	102	20.4	F
		20	4	4	0	83	20.7	F
		22	4	4	0	82	20.5	F
		23	5	5	0	107	21.4	F
		2	5	0	5	0	0	S
		3	3	2	1	9	3.0	S
		5	5	0	5	0	0	S
		6	5	3	2	8	1.6	S
		7	4	1	3	1	0.2	S
		10	5	1	4	2	0.4	S
		15	5	0	5	0	0	S
		16	5	0	5	0	0	S
		19	3	0	3	0	0	S
		21	5	4	1	15	3.0	S
E	8	1	4	4	0	72	18.0	F
		2	4	4	0	67	16.7	F
		4	3	3	0	49	16.3	F
		6	3	3	0	57	19.0	F
		3	3	1	2	1	0.3	S
		5	5	3	2	9	1.8	S
		7	3	0	3	0	0	S
		8	5	2	3	2	0.4	S
		9	3	0	3	0	0	S

TABLE V. *The Segregation Result in the Progenies of Self-compatible Plants in 1927*

Mother-plant No.	Number of plants: tested	self-compatible	self-incompatible	As 1:1: Deviation	Probable error
2.....	23	13	10	±1.5	±2.4
8.....	9	4	5	±0.5	±1.5
Total.....	32	17	15	±1.0	±2.8

TABLE VI. *The Result of Self-pollinations of the Progenies of Self-incompatible Plants in 1927*

Mother-plant Class	Mother-plant No.	Plant No. of the progeny	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Fertile or sterile
B	5	2	5	5	0	93	18.6	F
		1	3	0	3	0	0	S
C	6	1	5	1	4	2	0.4	S
		2	4	0	4	0	0	S
	1	1	4	3	1	17	4.2	S
A	3	1	5	1	4	1	0.2	S
		2	4	2	2	4	1.0	S
		3	5	0	5	0	0	S
	4	1	5	2	3	3	0.6	S
		2	4	1	3	3	0.7	S
	9	1	5	0	5	0	0	S

TABLE VII. *The Result of Cross-pollinations between different Plants of the Progeny of Plant 2*

Crossing: Pistillate plant	Crossing: Pollen plant	Number of flowers: tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Fertile or sterile
a) *Between different self-compatible plants*							
2– 1(F)[1]	2– 4(F)	3	3	0	55	18.3	F
2– 4(F)	2– 1(F)	4	4	0	75	18.7	F
2– 1(F)	2–12(F)	4	4	0	72	18.0	F
2–12(F)	2– 1(F)	5	5	0	81	16.2	F
2– 1(F)	2–14(F)	5	5	0	83	16.6	F
2–14(F)	2– 1(F)	3	3	0	51	17.0	F
2– 1(F)	2–23(F)	5	5	0	87	17.4	F
2–23(F)	2– 1(F)	5	5	0	126	25.2	F
2– 4(F)	2–12(F)	5	5	0	105	21.0	F
2–12(F)	2– 4(F)	3	3	0	53	17.7	F
2– 4(F)	2–14(F)	4	4	0	76	19.0	F
2–14(F)	2– 4(F)	5	5	0	81	16.2	F
2– 4(F)	2–23(F)	5	5	0	85	17.0	F
2–23(F)	2– 4(F)	5	5	0	81	16.2	F
2–12(F)	2–14(F)	4	4	0	73	18.2	F
2–30(F)	2–15(F)	4	4	0	81	20.2	F
2–23(F)	2–14(F)	3	3	0	57	19.0	F

(1) Brace shows reciprocal crossings. F within parentheses means self-compatible, and S self-incompatible.

TABLE VII. (*Continued*)

Crossing: Pistillate plant	Crossing: Pollen plant	Number of flowers: tested	Number of flowers: bearing seeds	Number of flowers: without seed	Total number of seeds obtained	Average number of seeds per flower	Fertile or sterile
b) *Between different self-incompatible plants*							
2- 2(S)	2- 6(S)	5	5	0	81	16.2	F
2- 6(S)	2- 2(S)	5	5	0	78	15.6	F
2- 2(S)	2-21(S)	5	5	0	88	17.6	F
2-21(S)	2- 2(S)	4	4	0	80	16.0	F
2- 6(S)	2-21(S)	5	2	3	22	4.4	S
2-21(S)	2- 6(S)	5	1	4	8	1.6	S
c) *Between self-compatible and self-incompatible plants*							
2- 1(F)	2- 2(S)	5	5	0	106	21.2	F
2- 2(S)	2- 1(F)	3	3	0	52	17.3	F
2- 1(F)	2- 6(S)	4	4	0	81	16.2	F
2- 6(S)	2- 1(F)	4	4	0	70	17.5	F
2- 1(F)	2-21(S)	5	5	0	94	18.8	F
2-21(S)	2- 1(F)	2	2	0	39	19.5	F
2- 4(F)	2- 2(S)	4	4	0	80	20.0	F
2- 2(S)	2- 4(F)	5	5	0	103	20.6	F
2- 4(F)	2- 6(S)	5	5	0	105	21.0	F
2- 6(S)	2- 4(F)	4	4	0	62	15.5	F
2- 4(F)	2-21(S)	4	4	0	63	15.7	F
2-21(S)	2- 4(F)	2	2	0	36	18.0	F
2-12(F)	2- 2(S)	3	3	0	48	16.0	F
2- 2(S)	2-12(F)	4	4	0	79	19.7	F
2-12(F)	2- 6(S)	2	2	0	33	16.5	F
2- 6(S)	2-12(F)	4	4	0	75	18.7	F
2-12(F)	2-21(S)	3	3	0	50	16.7	F
2-21(S)	2-12(F)	3	3	0	56	18.7	F
2-14(F)	2- 2(S)	4	4	0	75	18.7	F
2- 2(S)	2-14(F)	5	5	0	102	20.4	F
2-14(F)	2- 6(S)	3	3	0	52	17.3	F
2- 6(S)	2-14(F)	4	4	0	64	16.0	F
2-21(S)	2-14(F)	4	4	0	73	18.2	F
2-23(F)	2- 2(S)	3	3	0	75	25.0	F
2- 2(S)	2-23(F)	5	5	0	105	21.0	F
2-23(F)	2- 6(S)	3	3	0	60	20.0	F
2- 6(S)	2-23(F)	4	4	0	63	15.7	F
2-23(F)	2-21(S)	3	3	0	64	21.3	F

TABLE VIII. *The Result of Cross-pollinations between Plants of Different Families in 1927*

Crossing: Pistillate plant	Crossing: Pollen plant	Number of flowers: tested	Number of flowers: bearing seeds	Number of flowers: without seed	Total number of seeds obtained	Average number of seeds per flower	Fertile or sterile
a) *Between the descendant of Plant 1 and self-compatible descendants of Plant 2*							
1– 1(S)	2– 1(F)	4	4	0	69	17.2	F
2– 1(F)	1– 1(S)	5	5	0	112	22.4	F
1– 1(S)	2– 4(F)	5	5	0	84	16.8	F
2– 4(F)	1– 1(S)	5	5	0	90	18.0	F
1– 1(S)	2–12(F)	5	5	0	77	15.4	F
2–12(F)	1– 1(S)	5	5	0	94	18.8	F
1– 1(S)	2–14(F)	5	5	0	83	16.6	F
2–14(F)	1– 1(S)	5	5	0	75	15.0	F
1– 1(S)	2–23(F)	5	5	0	96	19.2	F
2–23(F)	1– 1(S)	5	5	0	123	24.6	F
b) *Between the descendant of Plant 1 and self-incompatible descendants of Plant 2*							
1– 1(S)	2– 2(S)	4	4	0	97	19.4	F
2– 2(S)	1– 1(S)	5	5	0	123	24.6	F
1– 1(S)	2– 6(S)	3	2	1	4	1.3	S
2– 6(S)	1– 1(S)	5	3	2	11	2.2	S
1– 1(S)	2–21(S)	5	4	1	15	3.0	S
2–21(S)	1– 1(S)	3	2	1	7	2.3	S

TABLE IX. *The Fertility under the Natural Condition of the Plants Used for the Experiment in 1928*

Plant No.	Number of fruits tested	Number of seeds: Total	Number of seeds: Per fruit
11	9	50	5.6
12	9	150	16.7
13	10	225	22.5
14	8	60	7.5
15	7	135	19.3
16	9	199	22.1
17	10	177	17.7
18	10	196	19.6
19	5	63	12.6
20	7	96	13.7

TABLE X. *The Result of Self- and Cross-pollinations in 1928*

Source of pollen (Plant No.)	Number of flowers tested	Number of flowers bearing seeds	Number of flowers without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 11 (♀)				
11 (selfed)	*10*	*8*	*2*	*23*	*2.3*	*41.1*
12	10	10	0	22	2.2	39.3
13	9	9	0	45	5.0	89.2
14	8	8	0	48	6.0	107.1
15	9	9	0	59	6.6	117.8
16	10	9	1	29	2.9	51.8
17	9	9	0	52	5.8	103.6
18	10	10	0	27	2.7	48.1
19	6	6	0	27	4.5	80.3
20	7	3	4	7	1.0	17.8
		Plant 12 (♀)				
11	10	5	5	8	0.8	4.8
12 (selfed)	*9*	*6*	*3*	*6*	*0.7*	*4.2*
13	10	10	0	163	16.3	97.6
14	10	10	0	120	12.0	71.9
15	3	3	0	45	15.0	89.8
16	10	8	2	51	5.1	30.5
17	10	10	0	208	20.8	124.5
18	8	8	0	78	9.7	58.1
19	10	10	0	231	23.1	138.3
20	10	8	2	16	1.6	9.6
		Plant 13 (♀)				
11	10	10	0	203	20.3	90.2
12	10	10	0	219	21.9	97.3
13 (selfed)	*10*	*8*	*2*	*28*	*2.8*	*12.4*
14	10	9	1	123	12.3	54.7
15	10	9	1	77	7.7	34.2
16	10	10	0	274	27.4	121.8
17	10	9	1	100	10.0	44.4
18	10	10	0	243	24.3	108.0
19	9	9	0	124	13.9	61.2
20	10	10	0	202	20.2	89.8

TABLE X. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	Number of flowers bearing seeds	Number of flowers without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 14 (♀)				
11	10	10	0	110	11.0	146.7
12	10	10	0	85	8.5	113.3
13	10	9	1	36	3.6	48.0
14 (selfed)	*10*	*7*	*3*	*33*	*3.3*	*44.0*
15	10	7	3	25	2.5	33.3
16	10	10	0	91	9.1	120.0
17	10	7	3	26	2.6	34.7
18	8	8	0	45	5.6	74.6
19	10	10	0	56	5.6	74.6
20	9	9	0	66	7.3	97.3
		Plant 15 (♀)				
11	4	4	0	76	19.0	98.4
12	10	10	0	228	22.8	116.1
13	9	9	0	163	18.1	93.8
14	10	10	0	183	18.3	94.8
15 (selfed)	*10*	*10*	*0*	*183*	*18.3*	*94.8*
16	10	10	0	226	22.6	117.1
17	9	9	0	214	23.8	123.3
18	9	9	0	182	20.2	104.7
19	9	9	0	191	21.2	109.8
20	10	10	0	229	22.9	118.7
		Plant 16 (♀)				
11	10	10	0	244	24.4	110.4
12	9	9	0	208	23.1	104.8
13	10	10	0	277	27.7	125.?
14	7	7	0	189	27.0	122.1
15	9	9	0	254	28.2	127.6
16 (selfed)	*10*	*10*	*0*	*224*	*22.4*	*101.4*
17	10	10	0	278	27.8	125.7
18	9	9	0	198	22.0	99.5
19	10	10	0	273	27.3	123.5
20	9	9	0	227	25.2	114.1

TABLE X. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	Number of flowers bearing seeds	Number of flowers without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 17 (♀)				
11	9	9	0	186	20.7	111.1
12	10	10	0	219	21.9	123.7
13	10	10	0	50	5.0	28.3
14	10	10	0	71	7.1	40.1
15	10	9	1	86	8.6	48.6
16	9	9	0	207	23.0	129.9
17 (selfed)	*10*	*10*	*0*	*40*	*4.0*	*22.6*
18	10	10	0	140	14.0	79.1
19	10	10	0	153	15.3	86.4
20	10	10	0	176	17.6	99.4
		Plant 18 (♀)				
11	10	10	0	257	25.7	131.1
12	10	10	0	221	22.1	112.8
13	10	10	0	274	27.4	139.8
14	10	10	0	252	25.2	128.5
15	10	10	0	274	27.4	139.8
16	10	10	0	254	25.4	129.6
17	10	10	0	242	24.2	123.5
18 (selfed)	*10*	*10*	*0*	*61*	*6.1*	*31.1*
19	10	10	0	229	22.9	116.8
20	10	10	0	255	25.5	130.1
		Plant 19 (♀)				
11	1	1	0	12	12.0	95.2
12	9	9	0	109	12.1	95.2
13	10	10	0	103	10.3	81.7
14	9	9	0	80	8.9	70.6
15	10	10	0	111	11.1	88.1
16	8	8	0	126	15.7	125.0
17	10	10	0	162	16.2	128.6
18	10	10	0	56	5.6	44.4
19 (selfed)	*9*	*7*	*2*	*51*	*5.7*	*45.2*
20	9	9	0	108	12.0	94.4

TABLE X. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 20 (♀)				
11	10	9	1	45	4.5	32.8
12	10	6	4	28	2.8	20.4
13	9	9	0	131	14.6	106.6
14	10	10	0	174	17.4	126.9
15	10	10	0	193	19.3	140.8
16	10	9	1	56	5.6	40.9
17	10	10	0	158	15.8	115.2
18	10	10	0	70	7.0	51.1
19	10	10	0	162	16.2	118.1
20 (selfed)	*10*	*5*	*5*	*10*	*1.0*	*7.3*

TABLE XI. *Variations of Matings in the Relative Fertility in 1928*

	Relative fertility (%)	Plant No. 11	12	13	14	15	16	17	18	19	20
Incompatible matings	0.1– 10.0		3								1
	10.1– 20.0	1		1							
	20.1– 30.0							2			1
	30.1– 40.0	1	1	1	2				1		1
	40.1– 50.0	2		1	2			2		2	1
	50.1– 60.0	1	1	1							1
	60.1– 70.0			1							
Compatible matings	70.1– 80.0		1		2			1		1	
	80.1– 90.0	2	1	1				1		2	
	90.1–100.0		1	2	1	4	1	1		3	
	100.1–110.0	2		1		2	2				1
	110.1–120.0	1			2	3	2	2	2		2
	120.1–130.0		1	1		1	5	1	3	2	1
	130.1–140.0		1						4		
	140.1–150.5				1						1

TABLE XII. *The Fertility under the Natural Condition in 1929*

Plant No.	Number of fruits tested	Number of seeds	
		Total	Per fruit
Family 11			
11– 1	10	42	4.2
11– 2	8	39	4.9
11– 3	10	103	10.3
11– 4	10	73	7.3
11– 5	10	62	6.2
11– 6	8	39	4.9
11– 7	10	107	10.7
11– 8	10	134	13.4
11– 9	9	84	9.3
Family 13			
13– 1	10	116	11.6
13– 2	10	219	21.9
13– 3	9	173	19.2
13– 4	10	44	4.4
13– 5	10	93	9.3
13– 6	7	60	8.6
13– 7	7	63	9.0
13– 8	10	121	12.1
13– 9	9	110	12.2
13–10	10	77	7.7
13–11	10	73	7.3
13–12	10	101	10.1
13–13	10	71	7.1
13–14	10	44	4.4
Family 14			
14– 1	10	75	7.5
14– 2	10	65	6.5
14– 3	10	97	9.7
14– 4	10	51	5.1
14– 5	10	72	7.2
14– 6	8	25	3.1
14– 7	7	38	5.4
14– 8	10	79	7.9
14– 9	10	80	8.0
14–10	10	80	8.0
14–11	9	52	5.8
14–12	10	83	8.3
Family 15			
15– 1	9	120	13.3
15– 2	10	175	17.5
15– 3	9	113	12.6
15– 4	10	112	11.2
15– 5	7	115	16.4
15– 6	9	240	26.7
15– 7	10	126	12.6
15– 8	8	102	12.7
15– 9	8	105	13.1
15–10	10	182	18.2
15–11	10	118	11.8
15–12	7	89	12.7
15–13	9	143	15.9
15–14	8	142	17.7
15–15	10	131	13.1
15–16	10	60	6.0
15–17	10	66	6.6
15–18	8	55	6.9
15–19	10	198	19.8
15–20	10	202	20.2
15–21	9	114	12.7
15–22	7	117	16.7
15–23	10	132	13.2
15–24	5	36	7.2
15–25	8	130	16.2
15–26	11	141	12.8
15–27	10	176	17.6
15–28	10	218	21.8
15–29	10	97	9.7
15–30	10	182	18.2
15–31	8	95	11.9
15–32	10	116	11.6
15–33	8	99	12.4
15–34	9	66	7.3
15–35	10	104	10.4
15–36	10	120	12.0
15–37	7	75	10.7
15–38	9	107	13.0
15–39	6	85	14.2
15–40	10	231	23.1
15–41	9	130	14.4
15–42	10	161	16.1
15–43	9	79	8.8
15–44	9	87	9.7
15–45	10	89	8.9
15–46	9	67	7.4
15–47	5	46	9.2

TABLE XII. (*Continued*)

Plant No.	Number of fruits tested	Number of seeds	
		Total	Per fruit
Family 15 (continued)			
15–48	10	122	12.2
15–49	7	90	12.9
15–50	9	36	4.0
15–51	10	193	19.3
15–52	8	116	14.5
15–53	9	34	3.8
15–54	10	131	13.1
15–55	9	85	9.4
15–56	10	221	22.1
15–57	8	143	17.9
15–58	8	111	13.9
15–59	10	49	4.9
15–60	9	111	12.3
15–61	8	125	16.2
15–62	6	42	8.2
15–63	9	89	9.9
15–64	10	65	9.9
15–65	8	63	7.9
15–66	9	130	14.4
15–67	8	62	7.7
15–68	6	74	12.3
15–69	10	56	5.6
15–70	9	99	11.0
15–71	10	181	18.1
15–72	8	193	24.1
15–73	8	77	9.6
15–74	7	105	15.0
Family 16			
16– 1	10	180	18.0
16– 2	10	247	24.7
16– 3	10	103	10.3
16– 4	9	173	19.2
16– 5	10	216	21.6
16– 6	10	204	20.4
16– 7	10	253	25.3
16– 8	10	183	18.3
16– 9	10	184	18.4
16–10	9	119	13.2
16–11	8	183	22.9
16–12	10	155	15.5
16–13	5	69	13.8
16–14	10	72	7.2

Plant No.	Number of fruits tested	Number of seeds	
		Total	Per fruit
Family 16 (continued)			
16–15	6	42	7.0
16–16	10	88	8.8
16–17	8	97	12.1
16–18	10	144	14.4
16–19	8	173	21.6
16–20	6	41	6.8
16–21	10	184	18.4
16–22	10	101	10.1
16–23	10	211	21.1
16–24	10	177	17.7
16–25	7	125	17.9
16–26	6	69	11.5
16–27	10	213	21.3
16–28	8	65	8.1
16–29	8	79	9.9
16–30	10	145	14.5
16–31	10	164	16.4
16–32	9	80	8.9
16–33	10	183	18.3
Family 17			
17– 1	10	30	3.0
17– 2	8	38	4.5
17– 3	10	53	5.3
17– 4	6	84	14.0
17– 5	10	66	6.6
17– 6	10	184	18.4
17– 7	8	40	5.0
17– 8	9	91	10.1
17– 9	6	16	2.7
17–10	10	63	6.3
17–11	10	52	5.2
17–12	6	35	5.8
17–13	5	37	7.4
17–14	9	49	5.4
17–15	9	91	10.1
Family 18			
18– 1	10	145	14.5
18– 2	10	77	7.7
18– 3	10	65	6.5
18– 4	8	47	5.9

TABLE XII. (*Continued*)

Plant No.	Number of fruits tested	Number of seeds		Plant No.	Number of fruits tested	Number of seeds	
		Total	Per fruit			Total	Per fruit
Family 18 (continued)				*Family 19*			
18– 5	8	85	10.6	19– 1	8	37	4.6
18– 6	9	94	10.4	19– 2	10	79	7.9
18– 7	9	47	5.2	19– 3	7	46	6.6
18– 8	8	87	10.9	19– 4	8	65	8.1
18– 9	8	76	9.5	19– 5	8	105	13.1
18–10	9	49	5.4	19– 6	8	73	9.1
18–11	10	76	7.6	19– 7	7	40	5.7
18–12	10	49	4.9	19– 8	6	25	4.2
18–13	5	20	4.0	19– 9	10	58	5.8
18–14	7	25	3.6				
18–15	8	29	3.6	*Family 20*			
18–16	9	63	7.0	20– 1	10	201	20.1
18–17	9	84	9.3	20– 2	10	184	18.4
18–18	10	79	7.9	20– 3	10	85	8.5
18–19	10	57	5.7	20– 4	6	24	4.0

TABLE XIII. *The Result of Self-pollinations of the Progeny of Plant 11*

Plant No.	Number of flowers			Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
	tested	bearing seeds	without seed			
11–1	6	3	3	4	0.5	11.9
11–2	7	1	6	1	1.4	28.6
11–3	4	4	0	25	6.2	60.2
11–4	5	0	5	0	0	0
11–5	6	0	6	0	0	0
11–6	6	0	6	0	0	0
11–7	6	2	4	7	1.2	11.2
11–8	5	4	1	11	2.2	16.4
11–9	5	0	5	0	0	0

TABLE XIV. *The Result of Self-pollinations of the Progeny of Plant 13*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
13– 1	5	5	0	19	3.8	33.9
13– 2	6	1	5	3	0.5	2.3
13– 3	6	0	6	0	0	0
13– 4	5	4	1	7	1.4	31.8
13– 5	6	6	0	46	7.7	82.8
13– 6	6	4	2	7	1.2	14.0
13– 7	6	2	4	4	0.7	7.8
13– 8	6	6	0	12	2.0	16.5
13– 9	6	4	2	9	1.5	12.5
13–10	5	5	0	25	5.0	64.9
13–11	5	5	0	34	6.8	93.2
13–12	5	5	0	18	3.6	35.6
13–13	3	3	0	17	5.7	80.3
13–14	5	5	0	17	3.4	77.3

TABLE XV. *The Result of Self-pollinations of the Progeny of Plant 14*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
14– 1	5	5	0	33	6.6	88.0
14– 2	4	4	0	9	2.2	33.8
14– 3	6	6	0	13	2.2	22.7
14– 4	6	1	5	1	0.2	3.9
14– 5	5	5	0	38	7.2	100.0
14– 6	5	4	1	4	0.8	25.8
14– 7	5	0	5	0	0	0
14– 8	5	5	0	34	6.8	86.1
14– 9	6	1	5	2	0.3	3.7
14–10	6	2	4	3	0.5	6.2
14–11	4	0	4	0	0	0
14–12	6	4	2	8	1.3	15.7

TABLE XVI. *The Result of Self-pollinations of the Progeny of Plant 15*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
15- 1	5	0	5	0	0	0
15- 2	5	5	0	62	12.4	70.9
15- 3	6	1	5	3	0.5	3.9
15- 4	6	1	5	1	0.2	1.8
15- 5	5	5	0	89	17.8	108.5
15- 6	6	2	4	4	0.7	2.6
15- 7	5	5	0	45	9.0	71.4
15- 8	6	6	0	60	10.0	78.7
15- 9	6	6	0	38	6.3	47.9
15-10	6	6	0	71	11.8	64.8
15-11	5	4	1	15	3.0	25.4
15-12	6	6	0	23	3.8	31.1
15-13	5	5	0	74	14.8	93.1
15-14	6	2	4	2	0.3	1.7
15-15	4	4	0	44	11.0	84.0
15-16	6	6	0	48	8.0	133.3
15-17	5	2	3	4	0.8	12.1
15-18	3	2	1	6	2.0	29.0
15-19	5	1	4	1	0.2	1.0
15-20	6	6	0	37	6.2	30.7
15-21	5	5	0	64	12.8	100.8
15-22	5	3	2	5	1.0	6.0
15-23	6	6	0	82	12.0	90.9
15-24	5	0	5	0	0	0
15-25	6	6	0	21	3.5	21.5
15-26	6	4	2	10	1.4	10.9
15-27	6	6	0	94	15.7	89.2
15-28	6	6	0	155	25.8	118.3
15-29	6	6	0	38	6.3	64.9
15-30	6	4	2	17	2.8	15.4
15-31	5	1	4	1	0.2	1.7
15-32	5	2	3	7	1.4	12.1
15-33	6	3	3	6	1.0	8.1
15-34	5	5	0	40	8.0	109.6
15-35	5	5	0	32	6.4	61.5
15-36	5	5	0	49	9.8	81.7
15-37	7	1	6	5	0.7	6.5
15-38	5	0	5	0	0	0
15-39	6	6	0	73	12.2	85.9
15-40	6	6	0	14	2.3	99.6
15-41	5	2	3	6	1.2	8.3
15-42	6	4	2	13	2.2	13.7

TABLE XVI. (*Continued*)

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
15–43	6	6	0	53	8.8	100.0
15–44	5	4	1	43	8.6	88.7
15–45	6	6	0	60	10.0	112.4
15–46	6	3	3	5	0.8	10.8
15–47	6	0	6	0	0	0
15–48	5	5	0	21	4.2	24.4
15–49	5	5	0	55	11.0	85.3
15–50	6	0	6	0	0	0
15–51	5	5	0	80	16.0	82.9
15–52	6	1	5	2	0.3	2.1
15–53	6	6	0	12	2.0	52.6
15–54	2	2	0	26	13.0	99.2
15–55	6	4	2	4	0.7	7.4
15–56	5	5	0	87	17.4	78.7
15–57	5	0	5	0	0	0
15–58	6	6	0	74	12.3	87.8
15–59	6	1	5	1	0.2	4.1
15–60	6	6	0	28	4.7	38.2
15–61	5	5	0	101	20.2	124.6
15–62	5	4	1	4	0.8	9.8
15–63	6	5	1	13	2.2	22.4
15–64	6	6	0	33	5.5	84.6
15–65	6	6	0	37	6.2	78.5
15–66	6	6	0	72	12.0	83.3
15–67	5	5	0	39	7.8	101.3
15–68	5	2	3	4	0.8	6.5
15–69	6	6	0	25	4.2	75.0
15–70	6	6	0	68	11.3	102.7
15–71	4	4	0	25	6.2	34.5
15–72	5	5	0	98	19.6	81.3
15–73	6	4	2	5	0.8	8.3
15–74	6	4	2	24	4.0	26.7

TABLE XVII. *The Result of Self-pollinations of the Progeny of Plant 16*

Plant No.	Number of flowers tested	Number of flowers bearing seeds	Number of flowers without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
16– 1	5	5	0	69	13.8	72.2
16– 2	5	3	2	3	0.6	2.4
16– 3	6	5	1	15	2.5	24.3
16– 4	5	4	1	5	1.0	5.2
16– 5	6	1	5	1	0.2	0.9
16– 6	5	5	0	82	16.4	80.4
16– 7	6	4	2	7	1.2	4.7
16– 8	6	6	0	102	17.0	92.9
16– 9	6	5	1	12	2.0	10.9
16–10	5	1	4	1	0.2	1.5
16–11	4	4	0	68	17.0	74.2
16–12	6	2	4	3	0.5	3.2
16–13	6	4	2	12	2.0	14.5
16–14	6	3	3	8	1.3	18.1
16–15	5	5	0	30	6.0	85.7
16–16	5	2	3	4	0.8	9.1
16–17	6	0	6	0	0	0
16–18	5	1	4	1	0.2	1.4
16–19	5	2	3	3	0.6	2.8
16–20	5	5	0	11	2.2	32.4
16–21	5	5	0	68	13.6	73.9
16–22	5	5	0	44	8.8	87.1
16–23	6	4	2	14	2.3	11.7
16–24	6	6	0	82	16.7	94.3
16–25	6	4	2	5	0.8	4.5
16–26	5	5	0	40	8.0	69.6
16–27	6	3	3	12	2.0	97.3
16–28	5	3	2	7	1.4	17.3
16–29	5	5	0	34	6.8	68.7
16–30	3	3	0	48	16.0	110.3
16–31	5	5	0	69	13.8	84.1
16–32	6	0	6	0	0	0
16–33	3	3	0	57	19.0	103.8

TABLE XVIII. *The Result of Self-pollinations of the Progeny of Plant 17*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
17– 1	6	1	5	1	0.2	6.7
17– 2	6	2	4	2	0.3	6.7
17– 3	6	1	5	1	0.2	3.8
17– 4	6	5	1	15	2.5	17.9
17– 5	5	5	0	21	4.2	63.6
17– 6	6	0	6	0	0	0
17– 7	5	2	3	2	0.4	8.0
17– 8	5	2	3	2	0.4	4.0
17– 9	6	2	4	2	0.3	11.1
17–10	6	0	6	0	0	0
17–11	4	4	0	20	5.0	96.1
17–12	6	0	6	0	0	0
17–13	6	0	6	0	0	0
17–14	3	1	2	3	1.0	18.5
17–15	5	2	3	2	0.4	4.0

TABLE XIX. *The Result of Self-pollinations of the Progeny of Plant 18*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
18– 1	6	6	0	30	5.0	34.5
18– 2	6	1	5	4	0.7	9.1
18– 3	6	2	4	2	0.3	4.6
18– 4	6	3	3	3	0.5	8.8
18– 5	6	6	0	22	3.7	34.9
18– 6	4	1	3	1	0.2	1.9
18– 7	6	2	4	2	0.3	5.8
18– 8	6	0	6	0	0	0
18– 9	6	4	2	11	1.8	18.9
18–10	6	3	3	4	0.7	13.0
18–11	6	0	6	0	0	0
18–12	6	0	6	0	0	0
18–13	6	4	2	8	1.3	0
18–14	5	0	5	0	0	0
18–15	5	0	5	0	0	0
18–16	6	1	5	1	0.2	2.9
18–17	6	2	4	8	1.3	14.0
18–18	5	0	5	0	0	0
18–19	6	0	6	0	0	0

TABLE XX. *The Result of Self-pollinations of the Progeny of Plant 19*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
19–1	5	5	0	18	3.6	78.3
19–2	5	0	5	0	0	0
19–3	4	4	0	24	6.0	90.9
19–4	5	4	1	13	2.6	32.1
19–5	4	0	4	0	0	0
19–6	5	4	1	9	1.8	19.8
19–7	2	2	0	9	4.5	78.9
19–8	4	3	1	8	2.0	47.6
19–9	5	5	0	30	6.0	103.4

TABLE XXI. *The Result of Self-pollinations of the Progeny of Plant 20*

Plant No.	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
20–1	6	5	1	12	2.0	9.9
20–2	4	1	3	1	0.2	1.1
20–3	5	0	5	0	0	0
20–4	6	0	6	0	0	0

TABLE XXII. *Variations of Plants in the Relative Fertility in Self-pollinations in 1929*

	Relative fertility (%)	Family No. 11	13	14	15	16	17	18	19	20
Self-incompatible plants	0	4	1	2	6	2	4	8	2	2
	0.1– 10.0		2	3	16	10	6	6		2
	10.1– 20.0	3	3	1	7	5	3	3	1	
	20.1– 30.0	1		2	5	1				
	30.1– 40.0		3	1	4	1		2	1	
	40.1– 50.0				1				1	
Self-compatible plants	50.1– 60.0				1					
	60.1– 70.0	1	1		3	2	1			
	70.1– 80.0		1		6	3			2	
	80.1– 90.0		2	2	10	4				
	90.1–100.0		1	1	7	3	1		1	
	100.1–110.0				4	1			1	
	110.1–120.0				2	1				
	120.1–130.0				1					
	130.1–140.0				1					
Total	Incompatible	8	9	9	39	19	13	19	5	4
	Compatible	1	5	3	35	14	2	0	4	0

TABLE XXIII. *The Result of Cross-pollinations between Different Plants of the Family No. 11*

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 11-1 (♀)				
11-2	5	1	4	1	0.2	4.8
11-3	4	4	0	14	3.5	83.3
11-4	5	2	3	3	0.6	14.3
11-5	4	4	0	12	3.0	71.4
11-6	5	4	1	15	3.0	71.4
11-7	5	5	0	13	2.6	61.9
11-8	3	3	0	8	2.6	61.9
11-9	3	3	0	9	3.0	71.4
		Plant 11-2 (♀)				
11-1	5	0	5	0	0	0
11-3	5	5	0	30	6.0	122.5
11-4	5	0	5	0	0	0
11-5	4	4	0	12	3.0	61.2
11-6	5	5	0	38	7.6	155.1
11-7	5	5	0	21	4.2	85.7
11-8	5	5	0	17	3.4	69.4
11-9	5	5	0	27	5.4	110.2
		Plant 11-3 (♀)				
11-1	5	5	0	78	15.6	151.5
11-2	5	5	0	89	17.8	172.8
11-4	5	5	0	65	13.0	126.2
11-5	5	2	3	3	0.6	5.8
11-6	5	5	0	34	6.8	66.0
11-7	5	5	0	30	6.0	58.3
11-8	4	4	0	33	8.2	79.6
11-9	5	5	0	42	8.4	81.6
		Plant 11-4 (♀)				
11-1	5	1	4	1	0.2	2.7
11-2	5	1	4	1	0.2	2.7
11-3	5	5	0	35	7.0	95.9
11-5	3	3	0	14	4.7	64.4
11-6	5	5	0	20	4.0	54.8
11-7	5	5	0	20	4.0	54.8
11-8	5	5	0	27	5.4	74.0
11-9	5	5	0	28	5.6	76.7

TABLE XXIII. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 11-5 (♀)				
11-1	5	5	0	35	7.0	112.9
11-2	5	5	0	41	8.2	132.3
11-3	5	5	0	19	3.8	61.3
11-4	3	3	0	18	6.0	96.8
11-6	5	5	0	21	4.2	67.7
11-7	5	5	0	23	4.6	74.2
11-8	5	5	0	20	4.0	64.5
11-9	5	5	0	22	4.4	71.0
		Plant 11-6 (♀)				
11-1	2	1	1	1	0.5	10.2
11-2	5	1	4	1	0.2	4.1
11-3	5	3	2	7	1.4	28.6
11-4	2	0	2	0	0	0
11-5	3	0	3	0	0	0
11-7	3	1	2	1	0.3	6.1
11-8	5	4	1	7	1.4	28.6
11-9	4	1	3	1	0.2	4.1
		Plant 11-7 (♀)				
11-1	5	5	0	5	1.0	9.3
11-2	5	5	0	16	3.2	29.9
11-3	5	3	2	9	1.8	16.8
11-4	5	4	1	11	2.2	20.6
11-5	5	0	5	0	0	0
11-6	5	2	3	3	0.6	5.6
11-8	5	3	2	3	0.6	5.6
11-9	5	4	1	15	3.0	28.0
		Plant 11-8 (♀)				
11-1	5	3	2	6	1.2	9.0
11-2	5	3	2	7	1.4	10.4
11-3	5	4	1	5	1.0	7.5
11-4	5	3	2	7	1.4	10.4
11-5	5	1	4	1	0.2	1.5
11-6	5	4	1	9	1.8	13.4
11-7	5	2	3	4	0.8	6.0
11-9	5	5	0	12	2.4	17.9

TABLE XXIII. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
Plant 11–9 (♀)						
11–1	5	4	1	8	1.6	17.2
11–2	5	1	4	2	0.4	4.3
11–3	5	2	3	2	0.4	4.3
11–4	5	4	1	11	2.2	23.6
11–5	5	1	4	1	0.2	2.1
11–6	5	1	4	1	0.2	2.1
11–7	5	2	3	2	0.4	4.3
11–8	5	1	4	1	0.2	2.1

TABLE XXIV. *The Result of Cross-pollinations between Different Plants of the Family No. 15*

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
Plant 15–1 (♀)						
15– 2	5	5	0	89	17.8	138.6
15– 3	5	4	1	10	2.0	15.0
15– 4	5	5	0	16	3.2	24.1
15– 5	5	5	0	70	14.0	102.3
15– 6	5	5	0	15	3.0	22.6
15– 7	5	5	0	67	13.4	100.8
15– 8	5	5	0	59	11.8	88.7
15– 9	5	5	0	72	14.4	108.3
15–10	5	5	0	48	9.6	72.2
Plant 15–2 (♀)						
15– 1	5	5	0	76	15.2	87.1
15– 3	3	3	0	33	11.0	62.9
15– 4	5	5	0	82	16.4	93.7
15– 5	4	4	0	80	20.0	114.3
15– 6	5	5	0	64	12.8	73.1
15– 7	5	5	0	87	17.4	99.4
15– 8	5	5	0	84	16.8	96.0
15– 9	5	5	0	64	12.8	73.1
15–10	5	5	0	90	18.0	102.9

TABLE XXIV. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
Plant 15–3 (♀)						
15– 1	5	2	3	2	0.4	3.2
15– 2	5	5	0	42	8.4	66.7
15– 4	5	1	4	4	0.8	6.3
15– 5	5	5	0	44	8.8	69.8
15– 6	5	4	1	15	3.0	23.8
15– 7	5	5	0	45	9.0	71.4
15– 8	5	5	0	37	7.4	58.7
15– 9	5	5	0	55	11.0	87.3
15–10	5	5	0	46	9.2	73.0
Plant 15–4 (♀)						
15– 1	5	2	3	5	1.0	8.9
15– 2	5	5	0	50	10.0	89.3
15– 3	5	4	1	16	3.2	28.6
15– 5	5	5	0	58	11.6	103.6
15– 6	5	4	1	8	1.6	14.3
15– 7	5	5	0	45	9.0	80.4
15– 8	5	5	0	45	9.0	80.4
15– 9	5	5	0	57	11.4	101.8
15–10	5	5	0	48	9.6	85.7
Plant 15–5 (♀)						
15– 1	5	5	0	56	11.2	68.3
15– 2	5	5	0	67	13.4	81.7
15– 3	5	5	0	59	11.8	72.0
15– 4	5	5	0	57	11.4	69.5
15– 6	5	5	0	50	10.0	61.0
15– 7	5	5	0	83	16.6	101.2
15– 8	5	5	0	64	12.8	78.0
15– 9	5	5	0	82	16.4	100.0
15–10	5	5	0	56	11.2	68.3
Plant 15–6 (♀)						
15– 1	5	3	2	8	1.6	6.0
15– 2	5	5	0	140	28.0	104.9
15– 3	5	3	2	6	1.2	4.5
15– 4	5	4	1	16	3.2	12.0
15– 5	5	5	0	117	13.4	87.6
15– 7	5	5	0	138	27.6	103.4
15– 8	5	5	0	152	30.4	113.9
15– 9	5	5	0	143	28.6	107.1
15–10	5	5	0	149	29.8	111.6

TABLE XXIV. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 15–7 (♀)				
15– 1	5	5	0	45	9.0	71.4
15– 2	5	5	0	74	14.8	117.5
15– 3	5	5	0	41	8.2	65.1
15– 4	4	4	0	42	10.5	83.3
15– 5	5	5	0	55	11.0	87.3
15– 6	5	5	0	47	9.4	75.0
15– 8	5	5	0	56	11.2	88.9
15– 9	5	5	0	66	13.2	104.7
15–10	5	5	0	68	13.6	107.9
		Plant 15–8 (♀)				
15– 1	5	5	0	45	9.0	70.9
15– 2	5	5	0	98	19.6	154.3
15– 3	5	5	0	49	9.8	77.2
15– 4	5	5	0	51	10.2	80.3
15– 5	5	5	0	83	16.6	130.7
15– 6	5	5	0	83	16.6	130.7
15– 7	5	5	0	99	19.8	155.9
15– 9	5	5	0	87	17.4	137.0
15–10	5	5	0	57	11.4	89.8
		Plant 15–9 (♀)				
15– 1	5	5	0	47	9.4	71.8
15– 2	5	5	0	96	19.6	149.6
15– 3	5	5	0	51	10.2	77.9
15– 4	5	5	0	53	10.6	80.9
15– 5	5	5	0	100	20.0	152.6
15– 6	4	4	0	40	10.0	76.3
15– 7	5	5	0	85	17.0	129.8
15– 8	5	5	0	80	16.0	122.1
15–10	5	5	0	57	11.4	87.0
		Plant 15–10 (♀)				
15– 1	3	3	0	39	13.0	71.4
15– 2	5	5	0	102	20.4	112.1
15– 3	5	5	0	67	13.4	73.6
15– 4	5	5	0	79	15.8	86.8
15– 5	5	5	0	110	22.0	120.9
15– 6	5	5	0	87	17.4	95.6
15– 7	5	5	0	97	19.4	106.6
15– 8	5	5	0	97	19.4	106.6
15– 9	4	4	0	85	21.2	116.5

TABLE XXV. *The Result of Cross-pollinations between Different Plants of the Family No. 18*

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 18–1 (♀)				
18– 2	5	5	0	22	4.4	30.3
18– 3	5	5	0	15	3.0	20.7
18– 4	5	4	1	5	1.0	6.9
18– 5	5	5	0	24	4.8	33.1
18– 6	5	5	0	22	4.4	30.3
18– 7	5	5	0	28	5.6	38.6
18– 8	5	5	0	22	4.4	30.3
18– 9	5	5	0	12	2.4	16.6
18–10	5	5	0	13	2.6	17.9
		Plant 18–2 (♀)				
18– 1	5	4	1	8	1.6	20.8
18– 3	4	2	2	2	0.5	1.5
18– 4	5	0	5	0	0	0
18– 5	5	0	5	0	0	0
18– 6	5	1	4	1	0.2	2.0
18– 7	5	5	0	13	2.6	33.8
18– 8	5	2	3	3	0.6	7.8
18– 9	5	0	5	0	0	0
18–10	5	1	4	2	0.4	5.2
		Plant 18–3 (♀)				
18– 1	5	0	5	0	0	0
18– 2	5	3	2	3	0.6	9.2
18– 4	5	2	3	4	0.8	12.3
18– 5	5	1	4	1	0.2	3.1
18– 6	5	2	3	2	0.4	6.2
18– 7	5	0	5	0	0	0
18– 8	5	0	5	0	0	0
18– 9	5	0	5	0	0	0
18–10	5	2	3	2	0.4	6.2
		Plant 18–4 (♀)				
18– 1	5	3	2	4	0.8	14.0
18– 2	5	1	4	1	0.2	3.5
18– 3	5	3	2	3	0.6	10.5
18– 5	5	3	2	4	0.8	14.0
18– 6	4	4	0	10	2.5	43.8
18– 7	4	3	1	6	1.5	26.3
18– 8	5	2	3	2	0.4	7.0
18– 9	5	1	4	1	0.2	3.5
18–10	5	5	0	9	1.8	31.6

TABLE XXV. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 18–5 (♀)				
18– 1	5	5	0	17	3.4	32.1
18– 2	5	5	0	19	3.8	35.8
18– 3	5	0	5	0	0	0
18– 4	5	2	3	2	0.4	3.8
18– 6	5	3	2	3	0.6	5.7
18– 7	5	1	4	1	0.2	1.9
18– 8	5	5	0	24	4.8	45.3
18– 9	5	0	5	0	0	0
18–10	5	4	1	6	1.2	11.3
		Plant 18–6 (♀)				
18– 1	5	3	2	8	1.6	15.4
18– 2	5	4	1	15	3.0	18.8
18– 3	5	2	3	2	0.4	3.8
18– 4	5	2	3	2	0.4	3.8
18– 5	5	1	4	2	0.4	3.8
18– 7	5	2	3	8	1.6	15.4
18– 8	5	3	2	23	4.6	44.2
18– 9	5	2	3	3	0.6	5.8
18–10	5	3	2	4	0.8	7.7
		Plant 18–7 (♀)				
18– 1	5	0	5	0	0	0
18– 2	5	3	2	5	1.0	19.2
18– 3	5	0	5	0	0	0
18– 4	4	2	2	2	0.5	9.6
18– 5	5	2	3	3	0.6	11.5
18– 6	5	2	3	8	1.6	30.8
18– 8	5	3	2	10	2.0	38.5
18– 9	5	0	5	0	0	0
18–10	5	1	4	1	0.2	3.8
		Plant 18–8 (♀)				
18– 1	5	0	5	0	0	0
18– 2	5	0	5	0	0	0
18– 3	5	0	5	0	0	0
18– 4	5	0	5	0	0	0
18– 5	5	1	4	1	0.2	1.8
18– 6	5	0	5	0	0	0
18– 7	5	2	3	5	1.0	9.2
18– 9	5	0	5	0	0	0
18–10	5	1	4	1	0.2	1.8

TABLE XXV. (*Continued*)

Source of pollen (Plant No.)	Number of flowers tested	bearing seeds	without seed	Total number of seeds obtained	Average number of seeds per flower	Relative fertility (%)
		Plant 18–9 (♀)				
18– 1	5	3	2	18	3.6	37.9
18– 2	5	4	1	15	3.0	31.6
18– 3	5	2	3	2	0.4	4.2
18– 4	5	2	3	8	1.6	16.8
18– 5	4	4	0	12	3.0	31.6
18– 6	5	4	1	12	2.4	25.3
18– 7	5	3	2	8	1.6	16.8
18– 8	5	5	0	21	4.2	44.2
18–10	5	3	2	8	1.6	16.8
		Plant 18–10 (♀)				
18–1	5	0	5	0	0	0
18–2	5	2	3	6	1.2	22.2
18–3	5	0	5	0	0	0
18–4	5	1	4	1	0.2	3.7
18–5	5	0	5	0	0	0
18–6	5	1	4	1	0.2	3.7
18–7	4	3	1	3	0.7	13.0
18–8	5	5	0	10	2.0	37.2
18–9	5	0	5	0	0	0

TABLE XXVI. *Variations of Matings in the Relative Fertility in Cross-pollinations in 1929*

	Relative fertility (%)	Family No. 11	15	18
Incompatible matings	0	5		22
	0.1– 10.0	20	5	29
	10.1– 20.0	8	3	15
	20.1– 30.0	6	4	5
	30.1– 40.0			15
	40.1– 50.0			4
Compatible matings	50.1– 60.0	3	1	
	60.1– 70.0	9	8	
	70.1– 80.0	8	16	
	80.1– 90.0	3	17	
	90.1–100.0	2	5	
	100.1–110.0		14	
	110.1–120.0	2	6	
	120.1–130.0	2	3	
	130.1–140.0	1	4	
	140.1–150.0		1	
	150.1–160.0	2	3	
	160.1–170.0			
	170.1–180.0	1		

TABLE XXVII. *Comparison between the Expected Results and the Actual Ones of the Segregations in 1929*

Class in 1928	Family No.	Self-compatible		Self-incompatible	Total
A	11	1		8	9
	20	0		4	4
B	13	5		9	14
	14	3		9	12
	17	2		13	15
C	19	4		5	9
Total		15		48	63
Expected as 1:3		15.75		47.25	63
Deviation			±0.75		
Probable error			±2.32		
D	18	0		19	19
Expected as 0:1		0		19	19
Deviation			0		
E	16	14		19	33
F	15	35		39	74
Total		49		58	107
Expected as 1:1		53.5		53.5	107
Deviation			±4.5		
Probable error			±3.49		

TABLE XXVIII. *Genetic Constitutions of the Members of the Family No. 11*

Genotype	Theoretical ratio	Class in Diag. 3	Number of plants		Deviation	Probable error
			Theoretical	Actual		
$S_1S_1T_1T_1$	1	C	1.7	3	+1.3	±0.8
$S_1S_1T_1T_2$	2					
$S_1S_1T_2T_2$	1	—	0.6	0	−0.6	±0.5
$S_1S_2T_1T_1$	2	D	1.1	1	−0.1	±0.7
$S_1S_2T_1T_2$	4	A	2.2	4	+1.8	±0.9
$S_1S_2T_2T_2$	2	—	1.1	0	−1.1	±0.7
$S_2S_2T_1T_1$	1	—	0.6	0	−0.6	±0.5
$S_2S_2T_1T_2$	2	—	1.1	0	−1.1	±0.7
$S_2S_2T_2T_2$	1	B	0.6	1	+0.4	±0.5

TABLE XXIX. *Genetic Constitutions of the Members of the Family No. 15*

Genotype	Theoretical ratio	Class in Diag. 4	Number of plants		Deviation	Probable error
			Theoretical	Actual		
$S_2S_2T_1T_1$	1	A	2.5	4	+1.5	±0.9
$S_2S_3T_1T_1$	2	C	5.0	5	0	±1.1
$S_3S_3T_1T_1$	1	B	2.5	1	−1.5	±0.9

TABLE XXX. *Comparison between the Variation in the Relative Fertility of all the Compatible Self-pollinations in 1929 and the Distribution of the Normal Curve*

Relative fertility	Distribution of normal curve	Actual frequency	Deviation	Probable error	Dev./P.E.
30.1– 40.0	0.1	0	−0.1	±0.2	0.5
40.1– 50.0	0.5	0	−0.5	±0.5	1.0
50.1– 60.0	2.3	1	+1.3	±1.0	1.3
60.1– 70.0	6.6	8	+1.4	±1.6	0.9
70.1– 80.0	12.7	12	−0.7	±2.1	0.3
80.1– 90.0	16.3	18	+1.7	±2.4	0.7
90.1–100.0	13.9	14	+0.1	±2.2	0.05
100.1–110.0	7.9	6	−1.9	±1.8	1.1
110.1–120.0	3.0	3	0	±1.1	0
120.1–130.0	0.7	1	+0.3	±0.6	0.5
130.1–140.0	0.12	1	+0.78	±0.22	3.5

TABLE XXXI. *Comparison between the Variation in the Relative Fertility of all the Compatible Cross-pollinations in 1929 and the Distribution of the Normal Curve*

Relative fertility	Distribution of normal curve	Actual frequency	Deviation	Probable error	Dev./P.E.
Less– 50.0	9.6	0	−9.6	±2.0	4.8
50.1– 60.0	8.7	4	−4.7	±1.9	2.5
60.1– 70.0	12.5	17	+4.5	±2.2	2.0
70.1– 80.0	15.6	24	+8.4	±2.5	3.4
80.1– 90.0	16.8	20	+3.2	±2.5	1.3
90.1–100.0	15.7	7	−8.7	±2.5	3.5
100.1–110.0	12.7	14	+1.3	±2.3	0.6
110.1–120.0	8.9	8	−0.9	±1.9	0.5
120.1–130.0	5.4	5	−0.4	±1.5	0.3
130.1–140.0	2.8	5	+2.2	±1.1	2.0
140.1–150.0	1.3	1	−0.3	±0.8	0.4
150.1–160.0	0.7	5	+4.3	±0.6	7.2
160.1–170.0	0.2	0	−0.2	±0.3	0.7
170.1–180.0	0.05	1	+0.95	±0.15	6.3

TABLE XXXII. *The Result of the Natural Pollination of the Plants Used for the Physiological Studies*

Plant name	Number of flowers tested	Number of ovules			% fertile ovules	
		Total	Grown to seed	Failed to seed	Average	Coefficient of variability
A	30	1,071	494	571	46.13±1.05	18.47±1.66
B	30	1,036	418	618	40.35±0.99	19.96±1.71
C	30	898	535	363	59.58±1.50	20.40±1.85

TABLE XXXIII. *The Average Fertility of the Flowers of which Stigmas were Removed at Different Hours after Pollination*

Hours when stigmas were removed after pollination	Number of flowers tested	Number of ovules: Total	Number of ovules: Grown to seed	Number of ovules: Failed to seed	% fertile ovules: Average	% fertile ovules: Difference from unremoved	Diff./P.E.
a) *Plant A, self-pollination*							
6	12	449	1	448	0.22±0.19	−20.88±1.87	11.2
12	12	451	0	451	0.00±0.00	−21.10±1.86	11.3
24	18	671	23	648	3.43±0.63	−17.67±1.96	9.0
36	17	623	23	600	3.69±0.88	−17.41±2.06	8.5
48	16	551	22	529	3.99±0.91	−17.11±2.07	8.3
72	17	612	67	545	10.95±1.51	−10.18±2.40	4.2
96	17	624	68	556	10.90±1.46	−10.20±2.36	4.3
Unremoved	24	801	169	632	21.10±1.86	—	—
b) *Plant A × Plant B, cross-pollination*							
6	17	580	4	576	0.69±0.43	−44.03±1.78	24.7
12	16	546	23	523	4.21±1.30	−40.51±2.13	19.0
24	19	654	165	489	25.23±3.10	−19.49±3.53	5.5
36	11	399	145	254	36.34±3.48	− 8.38±3.87	2.2
48	22	769	310	459	40.31±2.08	− 4.41±2.68	1.6
72	14	496	243	253	48.99±3.13	+ 4.27±3.56	1.5
96	15	520	213	307	40.96±1.83	− 3.76±2.49	1.5
Unremoved	22	720	322	398	44.72±1.69	—	—

TABLE XXXIV. *Comparison of Fertility in Self-, Cross- and Natural Pollinations on Plant A*

Comparison	Difference	Diff./P.E.
Selfing with natural pollination of A	−25.03±2.08	12.0
Selfing of A with crossing A × B	−23.62±2.51	9.4
Crossing A × B with natural pollination of A	− 1.41±1.99	0.7

TABLE XXXV. *The Average Fertility of the Flowers of Different Ages*

Plot No.	Age of flower when pollinated	Number of flowers tested	Number of ovules: Total	Number of ovules: Grown to seed	Number of ovules: Failed to seed	% fertile ovules: Average	% fertile ovules: Difference from Plot 6	Diff./P.E.
a) *Plant A, self-pollination*								
1	5 days before blooming	26	937	196	741	20.92±1.63	− 0.18±2.47	0.1
2	4 „ „ „	20	737	248	489	33.59±2.63	+12.49±3.22	3.9
3	3 „ „ „	37	1,279	409	870	31.98±1.33	+10.88±2.29	4.8
4	2 „ „ „	42	1,443	554	889	38.39±1.73	+17.29±2.54	6.8
5	1 „ „ „	33	1,131	322	809	28.47±2.23	+ 7.37±2.90	2.6
6	1st day of blooming...	24	801	169	632	21.10±1.86	—	—
7	2nd „ „ „ ...	24	805	180	625	22.36±2.20	+ 1.26±2.88	0.4
8	3rd „ „ „ ...	26	876	200	676	22.83±1.85	+ 1.73±2.62	0.7
9	4th „ „ „ ...	29	1,009	274	735	27.16±1.70	+ 6.06±2.52	2.4

TABLE XXXV. (*Continued*)

Plot No.	Age of flower when pollinated	Number of flowers tested	Number of ovules			% fertile ovules		
			Total	Grown to seed	Failed to seed	Average	Difference from Plot 6	Diff./P.E.
	b) *Plant B, self-pollination*							
1	5 days before blooming	6	198	21	177	10.61±1.26	+ 7.06±1.36	5.2
2	4 „ „ „	9	284	46	238	16.20±2.35	+12.65±2.41	5.2
3	3 „ „ „	27	1,122	254	868	29.64±1.89	+26.09±1.96	13.3
4	2 „ „ „	40	1,271	408	863	32.10±0.95	+28.55±1.08	26.4
5	1 „ „ „	32	1,073	260	813	24.23±1.65	+20.68±1.73	12.0
6	1st day of blooming...	32	1,127	40	1,087	3.55±0.52	—	—
7	2nd „ „ „ ...	31	1,134	39	1,095	3.44±0.47	− 0.11±0.70	0.2
8	3rd „ „ „ ...	25	874	36	838	4.12±0.80	+ 0.57±0.94	0.6
9	4th „ „ „ ...	31	1,025	66	959	6.44±0.66	+ 2.89±0.84	3.4
	c) *Plant C, self-pollination*							
1	5 days before blooming	11	302	24	278	7.95±1.16	+ 1.49±1.39	1.1
3	3 „ „ „	31	818	303	515	37.04±2.12	+30.58±2.25	13.6
4	2 „ „ „	46	1,178	534	644	45.33±1.70	+38.67±1.86	20.8
5	1 „ „ „	31	851	202	649	23.74±2.29	+17.28±2.41	7.2
6	1st day of blooming...	34	960	62	898	6.46±0.76	—	—
7	2nd „ „ „ ...	26	593	57	536	9.61±1.42	+ 3.15±1.61	2.0
8	3rd „ „ „ ...	38	1,157	230	927	19.88±1.86	+13.42±2.01	6.7
9	4th „ „ „ ...	20	482	120	362	24.90±3.23	+18.44±3.32	5.6
	d) *Plant A × Plant B, cross-pollination*							
1	5 days before blooming	23	815	327	488	40.12±1.47	−4.60±2.24	2.1
2	4 „ „ „	40	1,355	584	771	43.10±1.08	−1.62±2.33	0.7
3	3 „ „ „	20	706	353	353	50.00±1.74	+5.28±2.43	2.2
4	2 „ „ „	36	1,253	613	640	48.92±0.91	+4.20±1.92	2.2
5	1 „ „ „	19	642	281	361	43.77±1.38	−0.95±2.18	0.4
6	1st day of blooming...	22	720	322	398	44.72±1.69	—	—
7	2nd „ „ „ ...	20	758	346	412	45.65±2.12	+0.93±2.71	0.3
8	3rd „ „ „ ...	22	843	401	442	47.57±1.43	+2.85±2.21	1.3
9	4th „ „ „ ...	20	743	366	377	49.26±1.65	+4.54±2.36	1.9

TABLE XXXVI. *Comparison between the Fertility in the Self-pollination Made Two Days before Blooming and that in the Natural Pollination*

Name of plant	Difference from the natural pollination	Diff./P.E.	% for the natural pollination
A	− 7.74±2.20	3.5	83.2
B	− 8.25±1.37	6.0	79.6
C	−14.25±2.28	6.2	76.1

On the Significance of the Root-Nodules of *Coriaria japonica*, A. Gr. in the Nitrogen Nutrition of the Plant

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With Plate I and 2 Text-figures

(Received July 2, 1930)

Introduction

Among the non-leguminous plants *Alnus, Elaeagnus, Ceanotus, Myrica* and *Coriaria* are known to possess well characterized nodules on their roots. The nodules are perennial and branch dichotomously in all possible directions, finally developing into roundish aggregates of considerable size.

Owing to exhaustive cytological studies done by Shibata (1902),(1) and afterwards by the same author in collaboration with Tahara (1917),(2) it has been established that the microorganisms responsible for the nodule-formation of these non-leguminous plants are more or less distinctly of actinomycetous nature. Also Lieske (1924)(3) described, through his microscopical and cultural studies, the endophyte of the nodule of *Alnus* as belonging to Actinomycetes.

As to whether these non-leguminous plants provided with root-nodules are capable of utilizing the atmospheric nitrogen for their nutrition, the question seems to have been only partially and incompletely attacked, and up to the present time with none of these plants this ability has been proved with equal degree of certainty as is the case with the leguminous plants.

(1) Shibata, K., Cytologische Studien üb. d. endotrophen Mykorrhizen. Jahrb. f. wiss. Bot. **37** (1902), 668.

(2) Shibata, K. u. Tahara, M., Studien üb. d. Wurzelknöllchen. Bot. Magaz., Tôkyô **31** (1917), 158.

(3) Lieske, R., Morphologie u. Biologie d. Strahlenpilze. Leipzig (1924), 264–268.

The experiments done in this line by HILTNER with *Alnus* (1896)[1] and with *Elaeagnus* (1898)[2] are well known. He made it highly probable that these two plants are able to assimilate atmospheric nitrogen when possessing root-nodules. But as far as the data available are concerned, even in the case of these two plants, it seems to be desirable to have more convincing evidences before coming to any definite conclusion in this respect.

That *Coriaria* possesses nodules in question has been first discovered in our country and announced by SHIBATA and TAHARA in the paper quoted above. According to these authors the case in this plant might be regarded as the most typical of actinomycetous nodules of the non-leguminous plants. They recognized in the nodules of *Coriaria japonica,* A. GR. most distinctly characteristic features of the actinomycetous endophyte as well as the very high grade of anatomical differentiation of the symbiotic tissues, almost equal to that of the leguminous nodules. In view of these facts it was thought to be interesting to study the significance of the root-nodules of this plant in connection with the nitrogen nutrition, and under the guidance of Prof. K. SHIBATA the experiments to be described in the following lines were conducted.

Some Physiological Observations on the *Coriaria* Nodules

The plant in question which is a deciduous shrub, known for the violent poison contained in the fruits and other parts, is distributed on heaths, hills, dunes, etc. It shows vigorous growth both on dry and humid places. On the roots growing in porous sandy soils, especially on spots not far from the soil surface where the air gains easy access, a considerable development of nodules, mostly in aggregates which are not seldom of the size of a walnut, is perceived, but nodules are produced only scantily on those roots extending deep into the heavy or humid soil. Analogous facts are experienced when conducting cultural experiments. The roots of *Coriaria* plants grown in pots containing light sandy soil under a restricted supply of water possess many nodule-aggregates, but in heavy soils, or even in sand when richly watered, the formation of nodules becomes suppressed. It seems that an 'aerobic' condition is necessary for the development of the *Coriaria* nodules. In this respect the nodule of *Coriaria* resembles that of

(1) HILTNER, L., Üb. d. Bedeutung d. Wurzelknöllchen v. *Alnus glutinosa* f. d. Stickstoffernährung dieser Pflanze. Landw. Versuchs-Sta. **46** (1896), 153.

(2) HILTNER, L., Üb. Entstehung u. physiologische Bedeutung d. Wurzelknöllchen. Forstl.-naturw. Zeitschr. 7 (1898), 415.

the leguminous plants and differs from that of *Alnus* which should develop



Fig. 1. Nodule-aggregates on the roots of *Coriaria japonica*, A. Gr.
(From Shibata and Tahara, by the courtesy of the authors.)

equally well under water as in soil (Hiltner)[1]. (Cf. Text-fig. 1).

The samples of the *Coriaria* nodules collected at different times of the year were analyzed for some of its constituents. The results were as follows:—

Table I. The results of analysis of the *Coriaria* nodules
(In percentage of the dry matter)

Date of collection	Part analyzed	Total nitrogen	Protein nitrogen	Ether extractive matter	Starch	Soluble saccharide	Fibre	Ash
May 26	Younger part of nodules	2.15	2.00	12.92	30.55	11.08	10.90	3.45
(„ „	Root proper	1.15	0.90	6.16	26.48	—	38.10	3.30)
Oct. 10	Younger part of nodules	2.69	2.21	—	—	—	—	—
„ „	Older part of nodules	2.04	1.61	—	—	—	—	—

(1) (1896), loc cit.

From these data it is seen that the percentage content of nitrogen in the nodule is higher than that of the root proper, though it is not appreciably higher than that of the aerial organs. Nor as far as the present data are concerned there seems to exist any such remarkable seasonal fluctuation in the nitrogen contents of nodules as that is observed in the nodules of herbaceous Leguminosae. Starch existing as granules is readily recognized under the microscope (cf. SHIBATA and TAHARA[1]). The ether extractive matter consists chiefly of resinous substance.

The Sand-Culture Experiment I

Toward the end of May 1921 a wild growing seedling of *Coriaria japonica* was transplanted into quartz-sand mixed with nutrient salts free from nitrogen and contained in a WAGNER's pot. By the middle of June it was evident that it had rooted and instead of the old shoots that had wilted, new ones began to develop one after another. The plant then grew up without showing any sign of nutritive defect. For the sake of comparison two plantlets raised from seeds in the laboratory in August of the year were planted each in a pot containing the same culture-sand as the first one. They showed only retarded growth with signs of nitrogen-starvation (see below). In July of 1923 the plant with root-nodules, growing in the first pot, had seven shoots measuring 5 to 8 mm in diameter and 50 to 85 cm in height. The stub measuring only 5 mm in diameter when transplanted then measured 16 mm. The plants for the control had respectively one and two shoots measuring 10 to 15 cm. This remarkable difference in the growth cannot be accounted for merely by somewhat unequal ages. On up-rooting the control plants showed no nodule.

The Sand-Culture Experiment II

This experiment was started with a number of seedlings germinating simultaneously; they were grown in separate pots with the culture-sand free from nitrogen and on some of them root-nodules were induced to develop by inoculation, while many others were left free from any infection throughout the experiment. The striking difference in growth manifesting itself between these two groups showed again very conspicuously the significant rôle played by the root-nodules in the nitrogen nutrition of this plant.

The seeds were obtained at the end of June, 1921 from the fruits just ripening on wild growing *Coriaria* plants. On August 13 they were

(1) loc. cit.

sterilized by immersing in a solution of chloride of lime and placed in Petri dishes on moistened pure cotton for germination. On August 20, two germinating seeds were sown in each experimental pot containing purified quartz-sand either mixed with nutrient salts, excepting compound nitrogen, and distilled water ('no nitrogen' pots), or with distilled water only ('no manure' pots).(1) The quartz-sand here used had been purified by soaking in 10% hydrochloric acid for one week, and all the pots had been sterilized in an autoclave before sowing. On October 20 to the plants in four 'no nitrogen' pots was given for the purpose of inoculation a small quantity of the diluted mash of the *Coriaria* nodules prepared under a sterile condition. Neither within that year nor until the end of April of the next year (1922) any effect of the 'inoculation' was observed. They showed almost indiscriminately feeble growth. At the beginning of November, 1921, their stems measured in average 7.5 cm. in height. At the end of May, 1922, they had each two or three off-shoots that had developed newly in the spring, measuring in length 9 to 13 cm. The leaves assumed light green colour which, however, was tinged, especially in those situated at the lower parts of the stems, by red pigment prevailing in leaves and stems. In the general appearance sign of liveliness lacked; the growth seemed to have practically ceased. At the beginning of May 'inoculation' was repeated in the four pots.

At about this time it was found that a small nodule was developing on a *Coriaria* plantlet that was growing together with many others in a larger pot. These plantlets had germinated simultaneously with those in the experimental pots, and the larger pot contained the 'no nitrogen' sand and had been 'inoculated'. This plant with nodule did not then differ from the plants in the experimental pots in size and in other respects to any appreciable extent. So this young plant was transplanted in a pot prepared as the 'no nitrogen' pot in the same way as in the experiment series and its further growth was observed. At the beginning of July, this and two other young plants growing in one of the 'inoculated' 'no nitrogen' pots showed the leaves increasing gradually in green colour. As the time

(1) Each 'no nitrogen' pot had the following content:—

Purified quartz-sand	2.4 kg.	Distilled water	600 ccm.
	gr.		gr.
Given as solution: $Ca(H_2PO_4)_2.2Aq$	0.50	Given as mixed powder: $Ca_3(PO_4)_2$	1.25
KCl	0.30	$CaCO_3$	0.75
$MgSO_4$	0.10	$CaSO_4$	0.25
$FeCl_3$	trace	$Fe_3(PO_4)_2.8Aq$	0.25

proceeded in the summer, in all the plants excepting these three, leaves almost wilted away and fell at the lower half of the stems, and smaller stunted leaves developed in their stead; roots were reddish-brown-coloured, thin, long, woody, and crooked. The 'no nitrogen' pots showed no better growth in comparison with the 'no manure' pots.

In the above mentioned three plants the leaves, from foot to top of the stems, assumed dark green colour and appeared soft and fleshly; red pigment had entirely vanished from the leaves and stems. In the general aspect they appeared very lively and continued their growth, while the others had ceased to do so. On examining the roots it was found that they possessed respectively one nodule-aggregate of the size of a soybean seed. Some notion of the difference in growth existing between the plants with and those without nodules may be obtained from the photograph (Pl. I, Fig. 1), taken toward the end of October. In this photograph only the two plants in the pot No. 1 have nodules on their roots; all the pots, including No. 1, are of 'no nitrogen' excepting A, B and C which are of 'no manure'.

By the middle of March, 1923, the three plants with nodules had developed green-coloured lively buds, prior by two weeks or so to the other plants. The buds of the latter were red-coloured and dwarfish. At the end of July the three plants with nodules had respectively several off-shoots measuring over 40 cm in length and some other shorter ones, while the control plants, either in the 'no nitrogen' or in 'no manure' pots had respectively only two to four slender off-shoots measuring 8 to 15 cm.

As the result had become clear, the experiment was finished at the end of July; on up-rooting it was confirmed that those three plants showing vigorous growth had respectively one nodule-aggregate of the size of a small walnut, while the others not even a trace of a nodule.

The Sand-Culture Experiment III

As it was found, as one may see from the preceding experiment as well as from a number of cases not described here, rather difficult to induce the formation of nodules by the way of artificial inoculation on the roots of the *Coriaria* plants, it was so arranged in the experiment lasting 1926–27 to transplant seedlings already possessing root-nodules and those kept free from any such into the experimental pots and to compare their further growths.

Coriaria seeds produced in the preceding summer were sown on February 2, 1926, in two separate pots, both containing the soil brought from the

place where the *Coriaria* plants were growing wild, the one pot having been sterilized in an autoclave before sowing. Almost all the seeds in the two pots germinated at the beginning of April simultaneously. In the month of July it was found that on the roots of the plants growing in the pot not sterilized small nodules were developing, while none on the roots of the plants in the other pot.

On August 2, when no appreciable difference in growth between the two pots had still set in[1], some seedlings of equal size were selected from each of the two pots, and one plant each was planted in a 'no nitrogen' pot of 2 litres' content. Each 'no nitrogen' pot had here the following content:—

Purified quartz-sand		2.4 kg.	Distilled water		600 ccm.
Given as solution	$MgSO_4$	0.30	Given as mixed powder	$Ca_3(PO_4)_2$	2.50
	KCl	0.50		$CaCO_3$	1.50
	$Na_2HPO_4.12Aq$	0.80		$CaSO_4$	0.50
	$FeCl_3$	0.06		$Fe_2(PO_4)_2.8Aq$	0.50

The difference in the further growth in the 'no nitrogen' pots may be seen from the data given in Tab. II and the figures inserted p. 216 (Text-fig. 2) and in the plate (Pl. I, Fig. 2).

The signs of nitrogen-starvation were again observed in the plants without nodule. Referring to Tab. II and Pl. I, Fig. 2, it should be noted that Nos. 2 and 3 have nodules, the others none. All are 'no nitrogen' pots excepting No. 9, which had been such until May 25, 1927, but on and after

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(1) However, between these two pots the difference of growth became afterwards gradually evident; the plants in the sterilized pot showed increasing symptoms of nitrogen-starvation, while in those in the other pot having root-nodules the colour of leaves changed from yellowish green which had until then prevailed, into deep green with the sign of an increasing liveliness.

In the experiment now (June, 1930) in progress a similar phenomenon is observed. The *Coriaria* seedlings, germinating in the middle of April of this year, are growing in two kinds of nursery pots prepared as the above. Those growing in the sterilized pots are all free from nodule, while on the roots of those growing in the pots not sterilized, small nodules have become visible without exception. Many seedlings of the latter group just like those of the former group have still yellowish green or reddish tinged leaves, while in some of them leaf-colour has already changed into green. All the seedlings of the latter group have more roots than those of the former group.

From these facts it will be clearly seen that the beneficial effects of root-nodules on the host plant is not limited to the particular cases of experiments where 'no nitrogen' culture media artificially composed were used.



Fig. 2. The *Coriaria* plants with (left, No. 3) and without root-nodule (right, No. 20) grown in 'no nitrogen' culture sand for one year. (Aug. 2, 1927.)

TABLE II. Comparison of the Growth of the *Coriaria* Plants with and without Root-nodules in 'no Nitrogen' Culture-sand

Plant	Nodule		1926 Aug. 2	1926 Sep. 6	1926 Nov. 6(1)	1927 April 25(1)	1927 May 10	1927 May 25	1927 June 15	1927 July 5	1927 July 15(2)	1927 Aug. 2
No. 2	+	Longest shoot (cm)	10.0	12.0	13.0	4.5	11.0	13.5	22.0	38.0	47.0	47.5
		Number of shoots	1	1	3	3	3	6	7	8	10	10
No. 3	+	Longest shoot (cm)	9.7	10.5	13.0	4.2	10.4	12.5	15.5	27.5	37.0	38.5
		Number of shoots	1	1	2	3	3	5	7	10	11	11
No. 8	−	Longest shoot (cm)	9.0	9.0	10.0	4.1	8.5	9.5	10.0	10.0	10.0	10.5
		Number of shoots	1	1	2	1	1	2	2	3	3	2 (1 wilted)
No. 9	−	Longest shoot (cm)	9.0	10.0	10.6	4.3	6.0	6.5	8.5	13.5	14.0	15.0
		Number of shoots	1	1	1	3	3	$NaNO_3 \rightarrow$ 3	4	6	6	6
No. 20	−	Longest shoot (cm)	9.5	10.5	11.0	4.4	6.5	7.0	8.0	8.1	8.2	8.2
		Number of shoots	1	1	2	3	4	4	4	4	6	3 (3 wilted)

(1) By young plants off-shoots wilt in the winter and new ones are extended from the stubs in the spring.

(2) Cf. Pl. I, Fig. 2.

this day it was supplied when watering with a small quantity each of the nitrate of soda in solution. Beneficial effect caused by the procedure is to be seen apparently both from the table and the figure. The result would have been more conspicuous if the supply of this compound had been limited to the quantity which did not everfeed the plant. At first only a beneficial influence was observed, which became evident first of all in the greening of the leaves. The plant seemed to be envigorated daily and green-coloured buds came forth; but after June 15, on which day the total amount of the nitrate of soda given in the pot reached 0.1 gramme, the colour of the leaves began to fade somehow and the leaves newly developing were found a little smaller than the preceding ones. The plant seems to have been overfed with the compound already by this time[1], and when on July 13 a fresh supply of the compound was done, the plant was found after two days (on the morning of photographing) to be wilting somehow at the top. Means were taken to lower the concentration of this nitrogen compound in the pot without changing those of the other ingredients and taking care not to hurt the roots; this again envigorated the plant to a certain extent.

The difference existing between the *Coriaria* plants with and without nodules in the quantities of the total plant substance formed and of nitrogen accumulated in the plant bodies may be seen from the following table (Tab. III) which contains the results obtained with the plants No. 3 (with nodules) and No. 20 (without nodule), up-rooted on August 2, 1927, nearly 70 weeks after the germination, and just one year after the transplantation to nitrogen-free culture-sand (see Text-fig. 2). The nodule-aggregate of No. 3 situated at the foot of the stub measured $25 \times 22 \times 17$ mm.

It is seen that the plant with nodules formed 23 times as much plant material and accumulated 30 times as much nitrogen as the control plant.

Table III. Comparison of the *Coriaria* plants with and without nodules grown in nitrogen-free sand for one year

Plant	Total sum of the lengths of off-shoots	Fresh weight of plant	Air-dry weight of plant	Total nitrogen content
	cm	gr	gr	gr
No. 3 (with nodules)	225.5	32.85	9.51	0.1012
		host 31.17 nodules 1.68	host 9.10 nodules 0.41	host 0.0944 nodules 0.0068
No. 20 (without nodules)	68.5	1.35	0.42	0.0034

(1) Trial to raise the *Coriaria* plants in media supplied with adequate amount of nitrate of soda and other nitrogen compounds is now in progress.

The amount of the nitrogen in the latter is very small; almost all the nitrogen contained in the plant provided with nodules must have been derived from no other source than atmospheric nitrogen.

Summary and Conclusion

Through repeated experiments with *Coriaria japonica*, A. GR. it has been confirmed that this plant when possessing root-nodules is able to show vigorous growth and accumulate nitrogen in its body in the culture medium free from compound nitrogen, in which the control plant[1] without root-nodule shows only retarded growth with signs of nitrogen-starvation.

Thus it has been proved beyond doubt that the *Coriaria* plants when possessing nodules on the roots, but not otherwise, are capable to promptly utilize atmospheric nitrogen for its nutrition like the Leguminosae.

It is the pleasant duty of the author to take here the opportunity of expressing his sincere gratitude to Prof. Keita SHIBATA for suggesting him to conduct the present investigation, and for valuable advices given throughout the experiment, and to Prof. Hirotaro ANDO, Director of the Station for liberally affording necessary facilities for this experiment. Thanks are also due to Prof. B. MIYAZAWA, formerly Director of Kanagawa Prefectural Agric. Exp. Sta., for his kind helps given to the author in obtaining necessary plant materials at the start of the experiment.

EXPLANATION OF PLATE I

Figure 1. Growth of *Coriaria japonica* A. GR. in 'no nitrogen' (pots Nos. 1–9 & 11) and in 'no manure' (pots A–C) culture sand. Pots Nos. 1–4 were inoculated but only the two plants in pot No. 1 formed nodules. (Oct. 24, 1922.)

Figure 2. Growth of *Coriaria japonica* A. GR. in 'no nitrogen' culture-sand, excepting No. 9, which received $NaNO_3$ shortly before. Only Nos. 2 and 3 have nodules. (July 15, 1927.) (cf. Tab. II and Text-fig. 2.)

(1) The circumstance that this plant does never form root-nodules by cursory infection under the conditions prevailing in the laboratory made it very easy to keep the control culture utterly free from nodules throughout long periods, in which respect HILNER's experiments (loc. cit.) with *Alnus* and *Elaeagnus* seems not to be very satisfactory.

PLATE I



Fig. 1

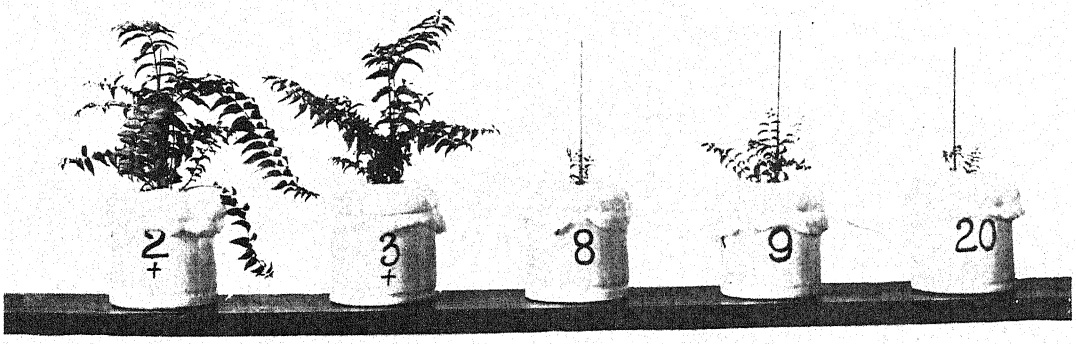


Fig. 2

Experimental Studies on Regeneration in *Bryophyllum calycinum*

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With 24 Figures in Text

(Received July 18, 1930)

Introduction

LOEB (1924) has pointed out two problems on regeneration as follows: "The first problem on regeneration is why does mutilation of an organism give rise to phenomena of growth which do not occur without mutilation, and second, why does the new growth frequently result in some kind of restoration of the old form of the mutilated organism".

In the present paper the writer deals with the problem cited above from LOEB's work, that is: What does induce regeneration in a separated leaf or stem? The writer has treated this problem rather experimentally in this paper, using *Bryophyllum calycinum* as an experimental material.

Theories on the cause of regeneration have been offered by many authors, for instance, by BONNET, SACHS, DE VRIES, ERRERA, GOEBEL, HABERLANDT and others. More recently LOEB has developed a theory on regeneration, using *Bryophyllum calycinum*. Employing sister leaves and pieces of stem of this plant as the experimental materials, LOEB found that the mass (dry weight) of the shoots and roots regenerated in isolated sister leaves and pieces of stem is directly proportional to that of the pieces from which they developed under equal environmental conditions and in the same duration. This relation in the regeneration is called by him the law of mass relation. From this law it follows, according to LOEB, that the regeneration is quantitatively determined by the mass of assimilated substances in stem or leaf. In other experiments LOEB pointed out the inhibiting effect in the regeneration

in *Bryophyllum calycinum*. Guided by these results of his experiments, LOEB concluded as to the cause of regeneration in *Bryophyllum calycinum*, that so long as the material which might be available for shoot and root formation in leaf is sent into the stem, no regeneration occurs, but when the flow of sap in leaf or stem is prevented partially or completely by isolation or other similar causes, regeneration takes place.

This conclusion has been met with both favourable and contradictory facts, for example, the results of the experiments of GOEBEL (1902), BRAUN (1918), CHILD and BELLAMY (1920), REED (1923), DOSTÁL (1927 & 1930), OSSENBECK (1927), KAKESITA (1928) and others. Though we already have valuable observations and experiments among them, the writer dared to carry out the present work, hoping that some contributions may be added by this work to the quantitative experiments in this field.

The Material

The material used in the present investigation is a tropical succulent plant, *Bryophyllum calycinum*. This plant consists morphologically of one straight unbranched stem possessing two leaves in each node. The two leaves in the same node are produced at the same time, therefore they are of the same age and develop symmetrically. Each pair is called sister leaves. The sister leaves are of the same size and same type. In greenhouse the leaf of this plant is thin between May and September, and in the rest period of the year thick and very fleshy. In the latter case it is called the winter leaf. So far as the leaf of this plant is attached to the stem, it performs the same functions as that of other common plants. But when it is isolated from the stem, it shows a strong power of regeneration. It is usual for this plant to be propagated in this way. The plants employed in the present investigation are those which the writer received from the Department of Horticulture of our University 6 years ago. They were propagated vegetatively by the writer in the greenhouse where some individuals produced flowers in February 1927 for the first time. It is common for this plant in the greenhouse to form flowers in February or March. However, in 1928 some plants flowered in November, and none of them produced flowers in February and March of the next year. The cause of this early formation of the flowers in that year is not clear. However, it seems probable to the writer that it was due to the extraordinarily long spell of fine weather during the summer and autumn of 1928. The necessity of sunlight, especially its ultraviolet ray, to the flower formation in this plant has been noticed by

LOEB in his book (1924). It may happen in this plant that at the time of flowering some leaves attached to the flowering plant secrete a clear sugary syrup from the leaf margin. So far as the writer's observation is concerned. the individuals which form flowers die soon after full flowering. The majority of the plants in the greenhouse do not produce any flower. The individuals, chosen for these experiments belong to this group. Only healthy and vigorous plants were used.

Regeneration in *Bryophyllum calycinum*

When a leaf, a notched piece of leaf or a piece of the stem of *Bryophyllum calycinum* is detached from the plant and put on moist sand, it regenerates a complete plant, i.e. roots and shoots. When a leaf is taken from the plant, root formation first occurs at the notches of the leaf about 5 days after isolation at the temperature of about 25°C. Soon after, shoot formation follows in the notches. However, regeneration in a piece of stem is somewhat different. If we detach a piece of the stem from the plant, shoot formation at the apical node begins to occur soon after the stem is isolated. But there is a relatively long latent period before the basal roots appear.

Now the question arises: Is it possible to induce such growth of new shoots and roots on stem or leaf without mutilation? To this question, LOEB answered affirmatively in his book (1924): When abnormal growth takes place in plant, e. g. when a stem contains many leaves and the stem growth is stopped, or when the sap flow has suffered, or when a plant becomes old, the regeneration takes place at the notches of the leaf which is still attached to the plant. Further, some others showed that such growth of new shoots and roots on the leaf or stem of normal and healthy plant of *Bryophyllum calycinum* can be brought about by giving it some particular treatment. CHILD and BELLAMY (1920) succeeded in producing roots and shoots at the notches of a leaf of a healthy plant of *Bryophyllum calycinum* by following certain procedures without taking off the leaf from the stem. The cooling of a certain zone of the petiole of this plant under the temperature of 2.5–4.0°C. for a few days, or pressing the petiole by a screw clamp to half its thickness is a very effective means of inducing the outgrowth of the leaf bud. In both cases, the treated leaf, the opposite untreated leaf, and often an adjoining untreated leaf show sign of development, when the blade is submerged under water. In their paper the authors have named this mode of procedure a physiological isolation. SMITH (1921) achieved to induce the bud development on attached leaves of *Bryophyllum calycinum* by

inoculation by *Bacterium tumefaciens*. He obtained also positive results in producing shoots and roots on stem, which forms one part of the whole plant, by making the crown gall by an injection of the same *Bacterium*. GOEBEL (1902) succeeded in producing roots and shoots at the notches of the attached leaves of a *Bryophyllum* plant by treating the whole plant body with ether vapour for 12–48 hours. This method of treatment has been already known as JOHANNSEN's "Ätherisieren" to force the bud development of a woody plant in the winter resting period. GOEBEL applied this treatment to regeneration in an attached leaf of *Bryophyllum* plant. REED (1920) obtained a positive result in inducing roots and shoots on an attached leaf of *Bryophyllum calycinum* by placing the whole plant body in a dark room. He placed this plant under natural conditions, and then deprived the plant of sunlight only. Two weeks later, regeneration at the notches of the attached leaves became visible. OSSENBECK (1927) attempted to induce the roots and shoots on the attached leaf of this plant by treating it by MOLISCH's warm-bath method, or by placing it within air rich in CO_2. In both cases, however, he obtained negative results.

Similar experiments were carried out successfully by the writer in following ways:

The writer placed twenty healthy potted plants about 1 year old in a cold glasshouse where the temperature was kept below that of the normal greenhouse, about 10°C. But other environmental conditions—light, aeration etc.—were almost equal to those in the normal greenhouse. About 3 weeks later, all individuals, which were placed in the cold glasshouse, formed vigorously roots and shoots on the attached leaves of the middle and lower part of the stem[1] (Fig. 1 and 2). In some plants, not only did the development of roots and shoots on attached leaves occur, but also the axillary bud in the node on the upper part of the stem began to develop (Fig. 3). After the development of the newly produced parts all plants were returned to the normal greenhouse (20°–30°C.). The mother plants and the newly regenerated



Fig. 1.

(1) OSSENBECK (1927) reported that in *Bryophyllum crenatum* and *Bryophyllum calycinum* the regeneration on attached leaves of the stem was induced by cooling the pots in which the plants were planted.

parts too grew normally and lived for a long time. This experiment was carried out from December 1928 to January 1929.

The writer has already reported (1928) that MOLISCH's warm-bath method was a very effective means of producing roots and shoots in the attached leaf of *Bryophyllum calycinum*. The result of the experiments, which will be described below, is that which was repeated by the writer during June and September 1928. In this experiment, the writer submerged almost all parts of the plant body in a warm bath of 30–35°C. for

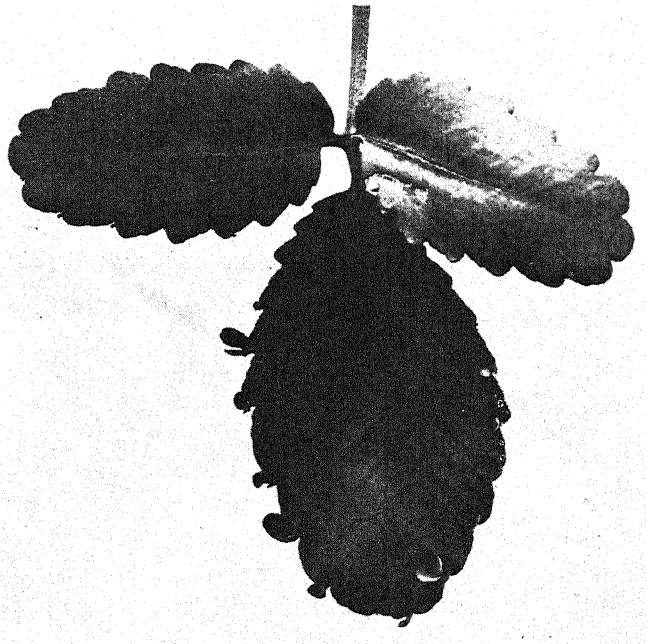


Fig. 2.

8 hours or more. Then the material used was placed in the greenhouse (about 20–30°C.). In the greenhouse, the treated plant was kept under normal conditions, namely, it was kept without either immersing the treated leaves in water or without covering the treated plant body with a bell jar. After about 5 days the treated leaves began to produce roots. This root formation occurred at first chiefly in the notches of the treated leaves which are attached to the upper part of the stem. After this happened, the leaves attached to the middle and lower part of the stem began to produce roots. Shoot formation took place also mainly on such leaves. Generally speaking,

the regenerated roots dried off soon after their appearance, though the regenerated shoots lived for a long time. In some cases, not only did the formation of roots and shoots on attached leaves occur, but also adventitious root production took place in the lower part of the stem. This observation was made from June to September 1928. The writer gained similar results by the following method. The potted plant was placed in a glass chamber in the laboratory. Then the temperature in the glass chamber was suddenly raised with an electric heater to about 35–38°C. for about 5 hours (from 10 a.m. to 3 p.m.) every day for four successive days. After doing so, the plant was returned to the greenhouse (20–30°C.) A few days later the leaves, which are attached to the middle and lower part of the stem, produced roots and shoots in the notches. This experiment was worked out in January 1929. Similar observations in the greenhouse were made by the writer on regeneration in attached leaves of *Bryophyllum crenatum*. The healthy potted plants of this species, about 15–30 cm. in height, were put in the greenhouse at the temperature of about 20°C. In early June 1929, the temperature in the environment rose suddenly, so that the greenhouse temperature reached sometimes about 35°C. All individuals of this plant in the greenhouse produced shoots and roots at the notches of the attached leaves and adventitious roots on the stem at the lower level of the plants. In the latter two cases, the high temperature may be the main factor to cause the regeneration in attached leaves of *Bryophyllum* plants; it seems, however, to the writer that in these cases desiccation also is concerned in the occurrence of regeneration besides high temperature.

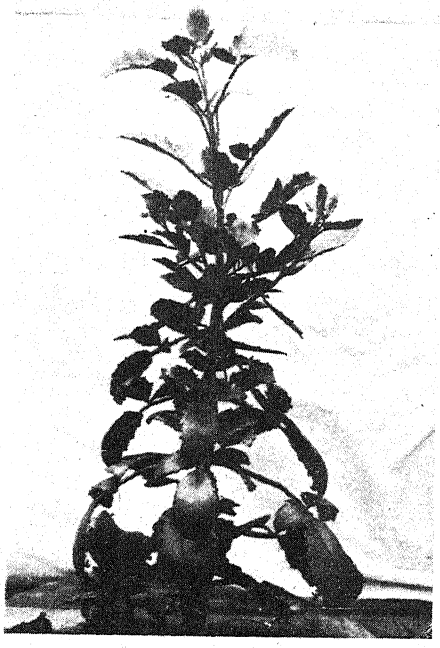


Fig. 3.

The writer (1928) already reported that by placing *Bryophyllum calycinum* under anaerobic condition for certain days, he succeeded in inducing root and shoot formation on attached leaves of the healthy plant. In the present investigation, the writer placed the potted plants of *Bryophyllum*

calycinum under anaerobic condition for 48 hours as described in the preceding report. After this treatment the plant materials were placed in the greenhouse (20–28°C.) and no bell jar was used to cover them. About 5 days later the regenerated roots appeared at the notches of the leaves of the treated plants except in very young leaves near the top; not long afterwards shoot formation occurred chiefly on the leaves which were attached to the middle and lower part of the stem. However, in this case the regenerated roots on the attached leaves dried off soon after their appearance. But new roots were formed after old ones at the same notches of the treated leaves. This repeated root formation on the treated leaves continued for sometime. Then it stopped and the regenerated roots disappeared. On the other hand, regenerated shoots, which were chiefly produced on the treated leaves of the middle and lower part of the stem, lived for a long time. This experiment was conducted in June 1928.

It is very reasonable to suppose that the above stated treatments which were carried out by many authors and the present writer to induce regeneration on attached leaves of healthy *Bryophyllum* plants, might have altered the metabolism in the plants.

Now as we have seen above, the "Ätherisieren", warm-bath treatment, introduction of anaerobic respiration, cooling and sudden rising of temperature under aerobic condition etc., are all effective means to produce roots and shoots on attached leaves of *Bryophyllum*. It is known that the "Ätherisieren" method is a common procedure which is used to break up the winter resting state of the bud. It is very interesting that other methods, which were attempted successfully by other authors in producing roots and shoots on attached leaves of *Bryophyllum calycinum*, are equal or very similar to the treatments designed to awake up the resting winter bud. What effect will be caused by these treatments upon the plant body? BORESCH (1924, 1926 and 1928) studied experimentally the forcing action of the warm-bath method and other similar treatments concerning their effect upon the development of the resting winter bud. From his experiments, BORESCH came to the following interesting conclusion. The warm-bath and other similar treatments cause the intramolecular respiration in bud. As a result of such metabolism, the intermediate and end products of alcoholic fermentation are accumulated in bud, of which, according to BORESCH, acetaldehyde works as a stimulant to force bud development. It is easy to think that the warm-bath and other similar treatments will have a similar influence upon *Bryophyllum* as well as upon the woody plants. That is, the treatments, which are used to force the resting winter bud, may cause just the same imper-

fect respiration in *Bryophyllum calycinum* as when this plant were treated with warm-bath, etc. Such imperfect respiration, which is caused by these treatments, may produce some chemical substances which might be concerned with the occurrence of regeneration in attached leaf of *Bryophyllum calycinum*. In order to confirm this, the experiment of injection with such substances as organic acids, aceton, pyruvic acid, acetaldelyde and ethyl alcohol which are assumed to be the products of intramolecular respiration, was carried out by using the attached leaves of *Bryophyllum calycinum* as material.

The method of injection is as follows: One pair of attached sister leaves on a healthy plant were selected out as material. One of these sister leaves was injected with any one of the above mentioned chemicals and the other with pure water as control. The injection was made in leaf blade near the notches. About 1–2 cc. of the preparation was injected with a common injection needle into the leaf. Care was taken to avoid injuring the vein of leaf with the injection needle. After injecting, the plant material was placed under normal conditions. All experiments in this series were made in the greenhouse.

Firstly we took ethyl alcohol for injection, because this substance is known as a product of anaerobic respiration in plant. 5% ethyl alcohol was prepared. One of the sister leaves was injected with 2 cc. of 5% ethyl alcohol once, and the other with the same amount of pure water as a control. After this treatment the plant was put under normal condition in the greenhouse of about 20–28°C. 2 days later the regenerated roots began to appear at the notches of the leaf which was injected with ethyl alcohol. Soon shoot formation also occurred at the same notches. On the control leaf such regeneration did not take place. After a few days from the beginning of development of the regenerated roots and shoots on the treated leaf, roots were merely dipped in water, in order to avoid the possibility of their drying off; the mother leaf and its regenerated shoots on the same notches were left in the air. The results, thus obtained, are shown in Fig. 4 and 5. This experiment was carried out in January 1929.

It is known that by intramolecular respiration various substances besides ethyl alcohol are produced in plant body, for instance, organic acids, aceton, methyl glyoxal, pyruvic acid, acetaldehyde etc. For this reason, we have chosen such substances as aceton, pyruvic acid, acetaldehyde and organic acids for injection too. 0.05% solutions of aceton, acetaldehyde and pyruvic acid were prepared. About 1 cc. of each preparation was injected into the leaf blade of one of the sister leaves which were attached to the middle level

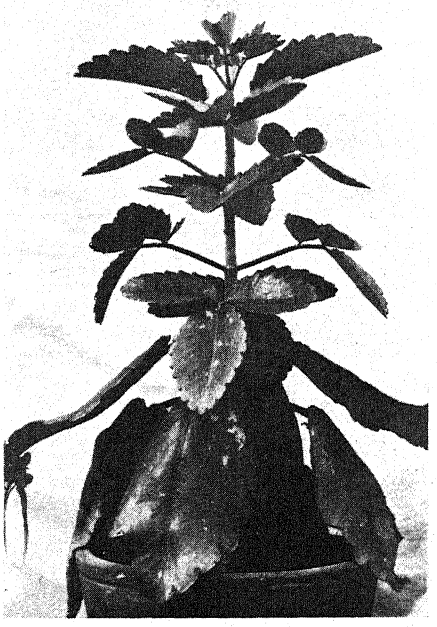


Fig. 4.

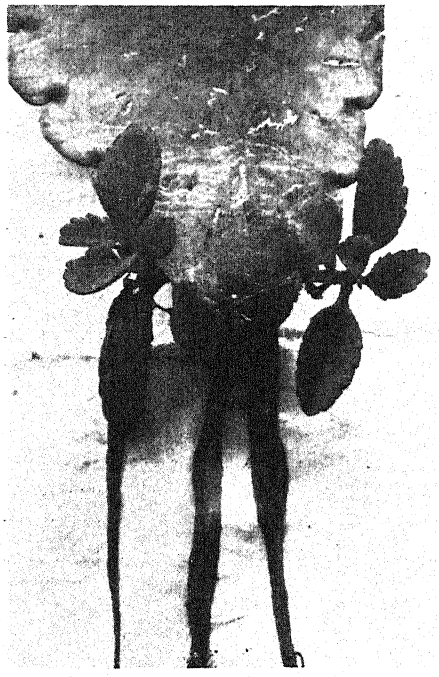


Fig. 5.

of a healthy plant. In every case, water injection was carried out on the opposite leaf as control. This experiment was done in January 1929. The results are given in the following table.

TABLE I

	0.05% aceton	Water as control	0.05% pyruvic acid	Water as control	0.05% acetaldehyde	Water as control
Regeneration	+ (Tiny Roots and Shoots)	−	+ (Tiny roots)	−	+ (Tiny roots)	−

Another experiment was made in which 5% acetaldehyde was used for injection. One of the sister leaves was injected with 5% acetaldehyde and another with water as control. A few days later some leaves attached near the leaf injected with acetaldehyde produced shoots and roots vigorously at the notches. At the same time, the leaf which was injected with 5% acetaldehyde decayed, owing perhaps to the high concentration of acetaldehyde. This experiment was made in February 1929.

The following preparations of organic acids were made for the purpose

of injection in the next experiment. 5 cc. of 0.1 M malic acid were mixed with various amounts of 0.1 N NaOH, and enough H_2O was added to bring this mixture to 10 cc. Then the following series were made:

	0.1 M malic acid	5 cc.	
No. 1	0.1 N NaOH	0	pH 2.4
	Water		
	0.1 M malic acid	5 cc.	
No. 2	0.1 N NaOH	1 cc.	pH 2.9
	Water	4 cc.	
	0.1 M malic acid	5 cc.	
No. 3	0.1 N NaOH	2 cc.	pH 3.4
	Water	3 cc.	
	0.1 M malic acid	5 cc.	
No. 4	0.1 N NaOH	3 cc.	pH 3.8
	Water	2 cc.	
	0.1 M malic acid	5 cc.	
No. 5	0.1 N NaOH	4 cc.	pH 4.3
	Water	1 cc.	
	0.1 M malic acid	5 cc.	
No. 6	0.1 N NaOH	5 cc.	pH 4.7
	Water	0	
Control	Pure water.		

The solutions No. 1, 2, 3, 4, 5 and 6 were injected into one of the two sister leaves of different individuals. The other of each pair sister leaves was injected with water as control. The solutions No. 1, 2, 3, 4 and 5 induced tiny roots in the notches of the injected leaves, though No. 6 solution did not cause regeneration. The control leaf showed no sign of such development of roots. These experiments were carried out from 14 April to 14 May 1928. Similar experiment was worked out with citric acid: 5 cc. of 0.1 M citric acid were mixed with 1 cc. of 0.1 N NaOH and 4 cc. of water. This solution had pH 2.8. It was injected into one of the two sister leaves and the other of the sister leaves with water as control. The notches of the leaf, which was injected with citric acid formed very tiny roots only. In the control leaf no regeneration occurred. This experiment was conducted from May 22 to 30, 1928.

Ethyl alcohol, aceton, pyruvic acid, acetaldehyde, and organic acids, which are assumed to be products of intramolecular respiration, are effective to induce regeneration in attached leaves of *Bryophyllum calycinum*. Among these substances, ethyl alcohol was found to behave most effectively in

this respect. From the experimental results of injection with end and intermediate products of intramolecular respiration, we now know that the products of intramolecular respiration play a stimulative rôle in the occurrence of regeneration in attached leaf. We may expect that the isolation of the leaf from the stem, which prevents direct flow of sap from the leaf into the stem and from the stem into leaf similarly, should cause in the isolated leaf metabolism which is quite different from that in the common normal attached leaf and the products in such case may stimulate the regeneration in isolated leaf. This was confirmed by the following experiments.

Changes in the pH and the Total Acidity in the Treated and Control Leaf of *Bryophyllum calycinum* during a Day

WARBURG (1886) claimed that in the normal attached leaf of *Bryophyllum calycinum* the acidity increases during the night and decreases as the time of day advances. Before WARBURG, HYNE, KRAUS, MEYER and others had already obtained similar results. More detailed investigation in this direction was carried out by GUSTAFSON (1925). In his work he studied not only the diurnal change of total acidity, but also that of pH in the leaf of *Bryophyllum calycinum*. The results of his experiments showed that both pH and total acidity increased steadily at night and reached the maximum at 10 o'clock in the morning and after that time both decreased to the minimum at about 4 o'clock in the afternoon and again increased until they reached the maximum in the following morning. Are or are not such changes in acidity during a day seen in isolated or otherwise treated leaf of *Bryophyllum calycinum*? To clear up this point, the writer carried out the following several experiments. The methods of experiment are as follows:

The leaf to be used as material was powdered in a morter by hand pressure, using approximately the same force each time. The paste of the leaf can be obtained in this way. This leaf paste was put on the filter paper, and filtered through it, drawing by the rotary pump, and using equal force each time. By these means a clear and colourless leaf sap can be obtained. If the sap is not clear even when it is obtained in this way, it is recommended to separate this dirt by centrifugal force. GUSTAFSON (1925) has shown that the centrifuging has no effect upon either the pH or the total acidity of the pressed out sap of this plant. Using the leaf sap, which was obtained by the methods stated above, the determination of total acidity and pH at various times during a day in isolated or otherwise treated

leaf was carried out. The pH was estimated by using CLARK and LUB's indicators and the total acidity by titration of 0.1 N NaOH. A sample of 10 cc. was used for each determination. All experiments were made on fine days during the period from March to August 1928. All experiments were conducted in the greenhouse.

Firstly the writer examined the change of acidity in the isolated leaf of this plant. For this purpose healthy, vigorous, about half a year old sister

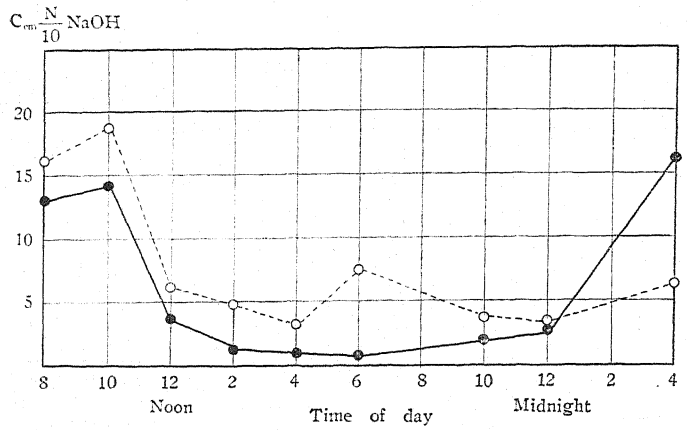


Fig. 6.
Changes of total acidity on 1st day after isolation.
----o---- represents the changes of acidity in isolated leaf.
——●—— represents the changes of acidity in control.

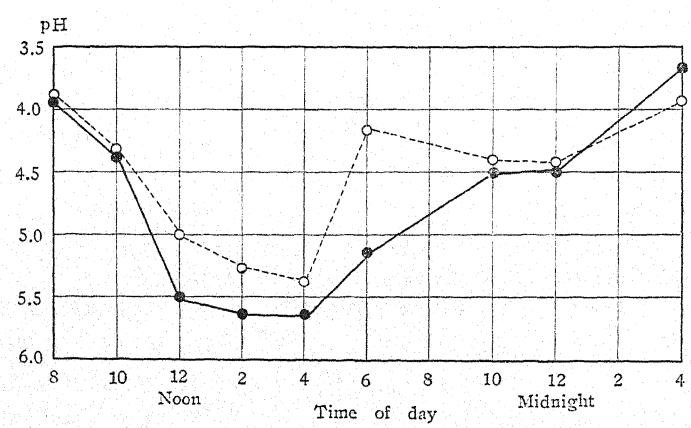


Fig. 7.
Changes of actual acidity on the 1st day after isolation.

leaves were chosen. One leaf of each pair was detached from the stem and put on moist soil in a pot, in which the mother plant was planted. The

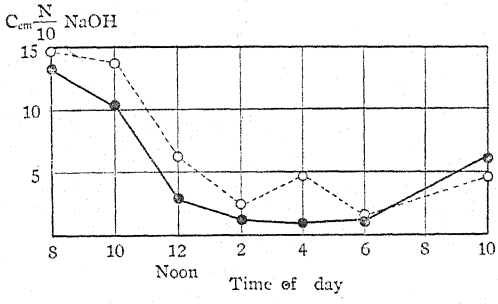


Fig. 8.
Changes of total acidity on the 2nd day after isolation.

other of each pair of sister leaves remained on the stem of the mother plant as control. Therefore, light and aeration etc. were almost equal in both isolated and control leaves. After a certain day, the determination was made by using the isolated and control leaves. The results of the experiment are represented in the graphs of Figs. 6–13.

In isolated leaves the acidity, generally speaking, is higher than in control, especially during the day time. This phenomenon is clearly seen during the first few days after isolation. It is known that the origin of organic acid in *Bryophyllum* is the in-

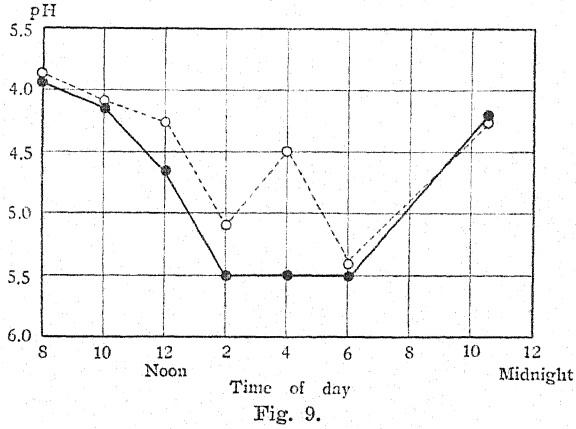


Fig. 9.
Changes of actual acidity on 2nd day after isolation.

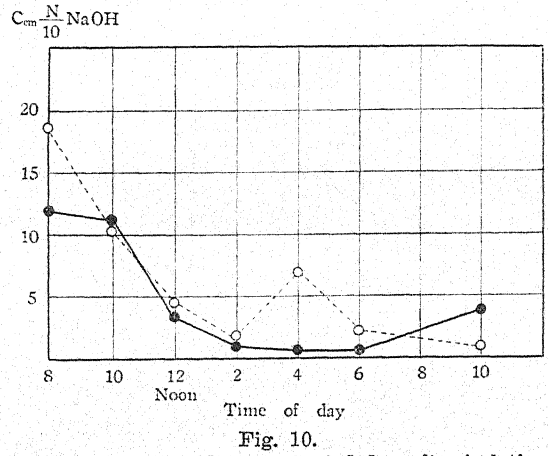


Fig. 10.
Changes of total acidity on the 3rd day after isolation.

complete oxidation of carbohydrate owing to incomplete respiration. According to this fact, respiration in an isolated leaf may be more incomplete than in a normal attached leaf, because the isolated leaf has a larger amount of acid as is shown in the above graphs. The variation in the curves of acidity during a day which is seen in the graphs may be

described as follows: In the attached control leaf, correlation exists between the pH and the total acidity as GUSTAFSON had already described in his paper (1925). Both the pH and total acidity show their highest level at about 10 o'clock in the morning and as the time of day advances both decrease to the lowest level in the afternoon. After that

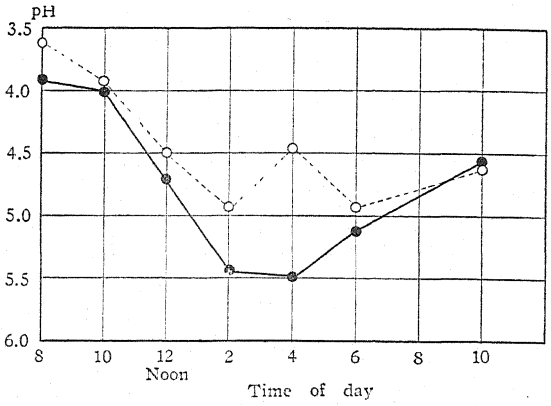


Fig. 11. Changes of actual acidity on the 3rd day after isolation.

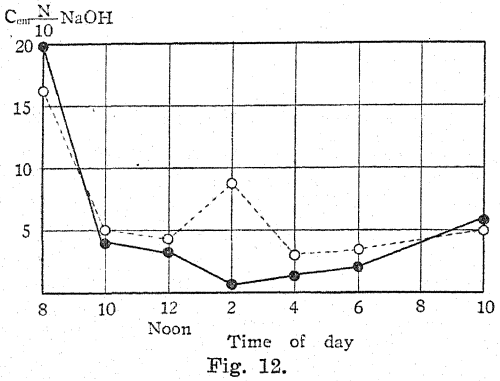


Fig. 12.
Changes of total acidity on the 4th day after isolation.

lowest point, both increase again until both reach the highest level in the next morning as GUSTAFSON (1925) had already pointed out in his paper. In the isolated leaf the parallelism between the pH and the total acidity is also seen. And the highest points in both are found in the morning, after which both decrease. However it is remarkable in the graph of the change of acidity in isolated leaf that at the time, in which the acidity in the control leaf shows almost its lowest level in the afternoon, both the pH and total acidity in the isolated leaf rise suddenly to a relatively high level and after that point both decrease again. And at night both the pH and total acidity in an

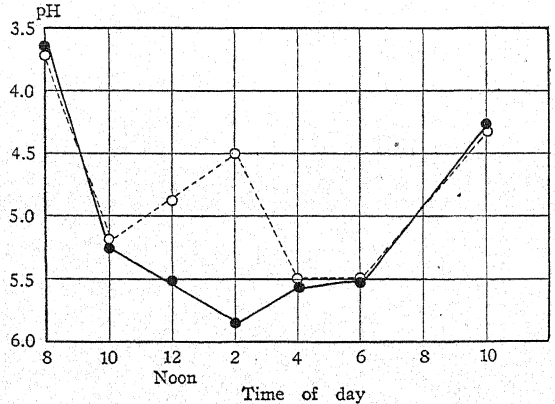


Fig. 13. Changes of actual acidity on the 4th day after isolation.

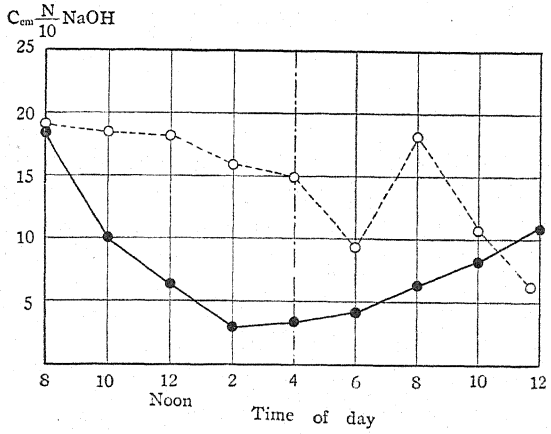


Fig. 14.
Changes of total acidity during and after warm bath treatment.
----○---- treated
——●—— control

isolated leaf steadily increase again until they reach the maximum in the next morning.

Other experiments were carried out in which healthy, vigorous, about one year old potted plants were taken as the materials which were divided into two groups. One group was immersed in a warm bath of about 35°C. for 8 hours from 8 o'clock in the morning to 4 o'clock in the afternoon. The other group served as a control. Measurements of acidity at various times during and after the warm bathing were made by using leaves attached to the middle part of the stem. Fig. 14–17 give the results of this experiment.

During warm bathing, both the pH and total acidity in treated leaves were very high and hardly any variation in the curve of acidity in the treated leaves was seen. This may be caused by the complete depression of oxygen respiration in the treated plant. Immediately after warm bathing, both pH and total acidity in treated leaves decreased suddenly and then rapidly increased, and then again decreased. On the second day after the experiment, the variation of acidity during a day in treated leaf was as follows: The maximum point of pH and total acidity were seen at about 10 o'clock in the morning, and then both

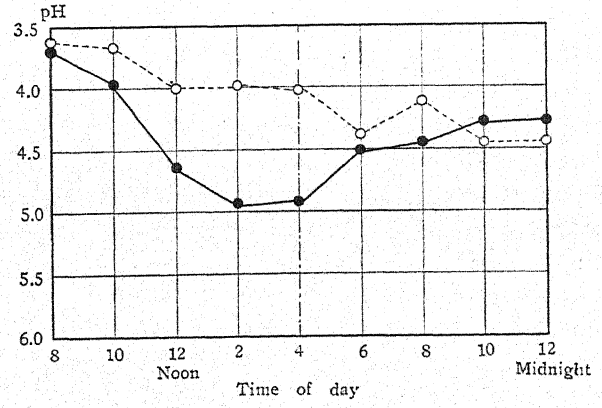


Fig. 15.
Changes of actual acidity during and after warm bath treatment.

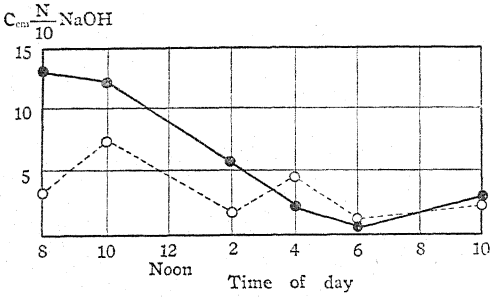


Fig. 16.
Changes of total acidity on the 2nd day after warm bath treatment.

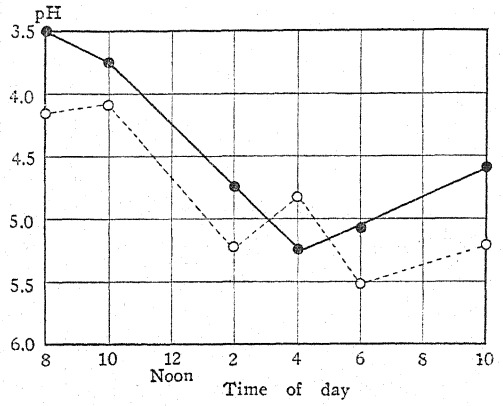


Fig. 17.
Changes of actual acidity on the 2nd day after warm bath treatment.

is similar to that in isolated leaf.

Another experiment was conducted in which healthy one year old plants were used as material. They were divided into two groups, in one of which each plant was covered with a bell jar, and air was replaced by H_2-gas. Into the jar a stream of fresh H_2-gas was constantly passing. The plants were kept steadily decreased to the minimum in the afternoon. At 4 o'clock p.m. both suddenly rose once, and then decreased again. At night both increased slowly. In the case of warm bath treatment, parallelism between pH and total acidity was also seen in treated leaves. On the second day after the experiment, the acidity in treated leaves, generally speaking, was lower than in the control. This may be due to the following reason: A considerable amount of assimilated substances is utilized for anaerobiosis, when the plant is put in warm bath. Hence, the substances which are available for formation of acid in plant body also decrease. This causes the acid in treated leaves for a few days after warm bathing to be less than in control. The type of curve in these cases

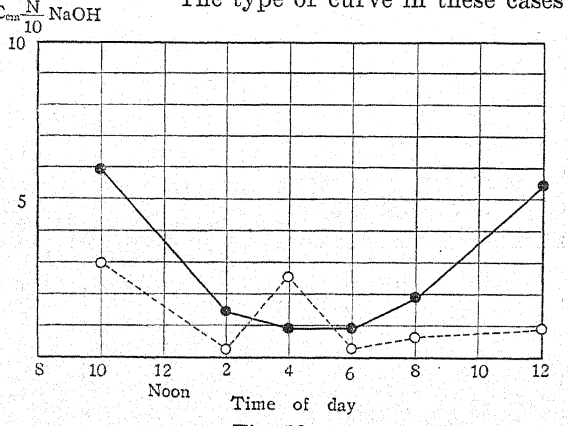


Fig. 18.
Changes of total acidity after introducing anaerobic respiration.

in this state for 48 hours. After this treatment, determination of acid in the leaf was made for a certain number of successive days. Another group served as the control. The leaves to be used for determination were those which were attached to the middle part of the stem of the plants. The results, which will be described below, are those obtained from 21 to 26 June 1928. The plant materials were put under anaerobic condition for the first 2 days of the experiment, namely, from 21 to 23 June. Then the treated plants were returned to normal aerobic condition at 10 a.m. 23 June. After this time, the estimation was carried out successively for 4 days by using the treated and control leaves. The results, which were obtained on 23 June, are given in Fig. 18 and 19.

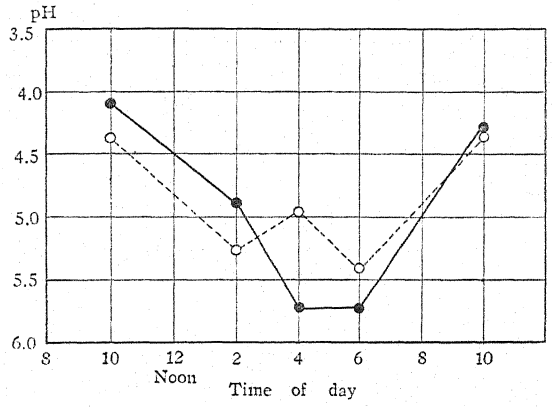


Fig. 19.
Changes of actual acidity after introducing anaerobic respiration.

The graphs in Figs. 18 and 19 show that when the plants, which were placed under anaerobic state for certain hours, were returned to aerobic state, the acidity in treated leaves suddenly decreased and then increased considerably and again decreased, as was seen in the case of warm bath treatment. This phenomenon may be connected with respiration in plant. This will be confirmed by experimental results which will be described in later pages. On the whole, the acidity in treated leaf after introducing anaerobic respiration is lower than in the control. This phenomenon may be equally explained as in the case

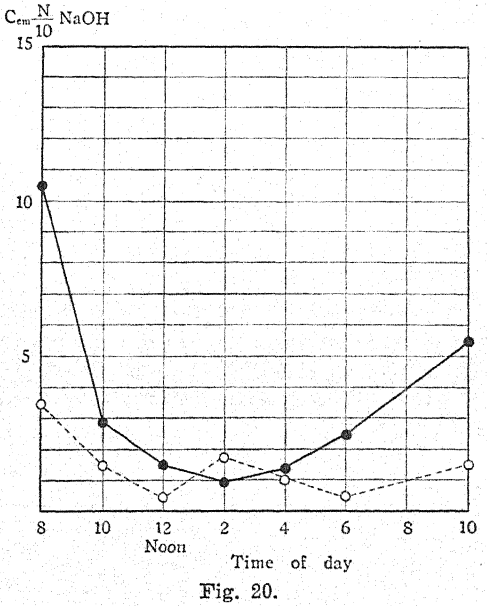


Fig. 20.
Changes of total acidity on the 2nd day after introducing anaerobic respiration.

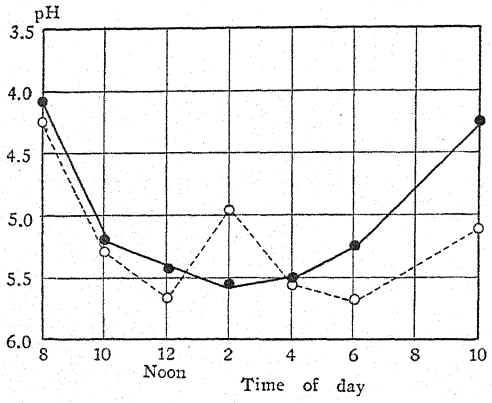


Fig. 21.
Changes of actual acidity on the 2nd day after introducing anaerobic respiration.

of warm bath treatment. Correlation between pH and total acidity is also seen in leaf sap after introducing anaerobic respiration. After isolation, after warm bathing, or after introduction of anaerobic respiration, the metabolism of acid during one day is exactly the same.

In connection with metabolism of acid in *Bryophyllum* some related experiments, which were carried out by the writer, will be described hereunder.

According to KRAUS and others, the organic acid in succulent plants is mainly malic acid. Some organic acids in the expressed juice of leaf were examined. Malic acid was detected by the colour reaction, using mixture of α- or β-naphtol and H_2SO_4 or resorcin and H_2SO_4. Citric acid was detected by DEINIGE's reaction. Oxalic acid was proved by the production of precipitation of Ca-oxalate. Tartaric acid was detected by MÖHLER's reaction. Fumaric acid was tested by the colour reaction, using mixture of α-naphtol and H_2SO_4. The results are as follows:

TABLE II

	Malic acid	Citric acid	Oxalic acid	Tartaric acid	Fumaric acid
Isolated leaf	+	−	−	−	−
Attached leaf	+	−	−	−	−

Another experiment was made by the writer for estimating the buffer action of sap in isolated and attached leaves of *Bryophyllum calycinum* at various times during a day. GUSTAFSON (1925) examined the buffer action in the expressed juice of *Bryophyllum* leaf on the alkaline side. In this experiment, the writer examined the buffer action in leaf sap of this plant on the acid side. Two and a half cc. of sample were taken and various amounts of 0.1 N HCl added to it. Enough H_2O was added to bring this mixture to 5 cc. The titration curves were obtained (Fig. 22).

Next, the writer carried out the estimation of pH in the notch of isolated leaf of *Bryophyllum calycinum*, according to the methods and technique of SMALL (1926).

In the notches of isolated leaf 4 days after isolation, pH was 5.2—4.0.

Further, the writer attempted to estimate pH in the portions of the same leaf other than in the notches, using exactly the same methods and technique. The result was the same as in the case of the notches: pH 5.2—pH 4.0.

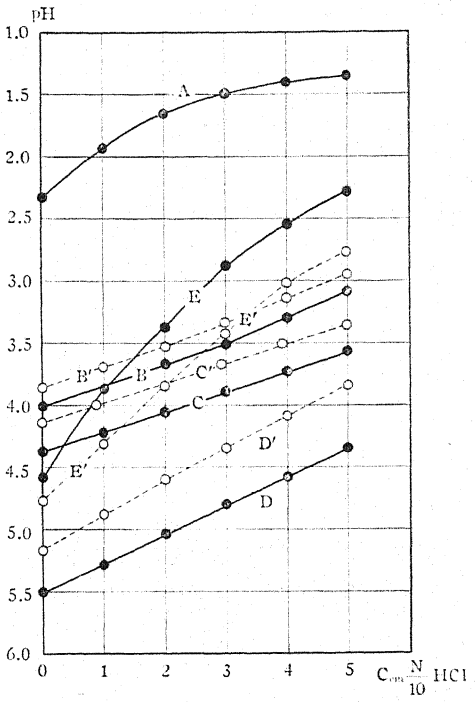


Fig. 22.

Titration curve,
A,.....M/10 Malic acid;
B, B′,.....E.E′, expressed juice of *Bryophyllum* leaf;
B, C, D, E, attached leaf (control);
B′, C′, D′, E′, isolated leaf;
BB′, juice collected 8 a.m.;
CC′, juice collected 12 a.m.;
DD′, juice collected 4 p.m.;
EE′, juice collected 10 p.m.

Estimation of the Volume of CO_2 Output from the Treated and Control Leaf and from the Stem of *Bryophyllum calycinum*

In these experiments, utmost care must be taken for selecting out the material. In this experiment, about one year old, healthy and vigorous plants were taken as the material. When the leaf was to be used as the material, the thick winter leaf only was selected throughout this work.

Measurement of the volume of CO_2 formed was done by absorption in baryta water. The titration of baryta water was made with oxalic acid. 100 cc. baryta water was put into a PETTENKOFER's tube. One end of the tube was connected with plant container, into which CO_2-free fresh air only came constantly. The other end of the PETTENKOFER's tube was connected with an air pump, by means of which a uniform air stream from the plant container was maintained through the PETTENKOFER's tube. 1000 cc. air stream were passed per hour constantly through the PETTENKOFER's

tube during the experiment. For the determination of CO_2 output from the leaf, a leaf chamber was used. Its structure is as follows:

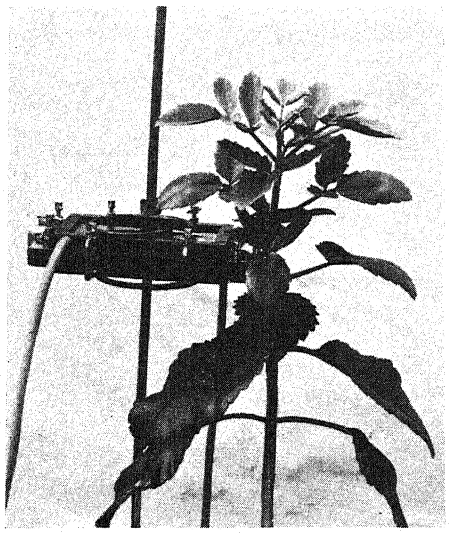


Fig. 23.

The leaf chamber (Fig. 23) was a wooden frame 15 cm. by 8 cm., and about 250 cc. capacity. This leaf chamber consisted of two equal boxes, each having a glass plate at the base which was connected with the wooden frame by means of putty. The putty was used to coat also the inner side of the wooden part of the boxes. The two boxes, which were fitted together, were connected tightly with each other by screw clamps when the experiment was carried out. Between the two boxes there was a heavy rubber sheet in order to avoid the entrance of air. The leaf chamber was held in position by a stand and one leaf was closed within it when the measurement was made (Fig. 23).

During experiments, the leaf chamber, in which the material was closed, was covered with a wooden plate, in order to keep the chamber under constant shade. All experiments in this series were carried out on five days between November 1928 and June 1929. The experiments were worked out in the greenhouse.

A healthy, vital, about half a year old leaf which is attached to the middle part of the stem, was selected out for the material. This leaf was closed within the leaf chamber, without being taken off from the stem, and the volume of CO_2 emission from it was measured from 8 a.m. to 11 p.m. in one day. The result was as follows:

TABLE III

	CO_2 formed (8 a.m.–1 p.m.)	CO_2 formed (1 p.m.–6 p.m.)	CO_2 formed (6 p.m.–11 p.m.)	Total CO_2 formed
Attached leaf	10. cc.	11.0 cc.	7.5 cc.	28.5 cc.
Temperature in greenhouse during experiment	23–25°C	25–28–20°C	20°C	

This experiment was conducted on 1 March 1929. The result will be used as the control for the following experiments, in which the leaf used for the experiment described above was detached from the stem and closed within the leaf chamber, and the determination of CO_2 emission from this isolated leaf was made for 4 successive days after isolation. The leaf was detached from the plant at 8 a.m. on the 1st day of the experiment (2 March) and immediately after isolation the measurement was commenced. The results, obtained on the 1st day after isolation, were as follows:

TABLE IV

	CO_2 formed (8 a.m.–1 p.m.)	CO_2 formed (1 p.m.–6 p.m.)	CO_2 formed (6 p.m.–11 p.m.)	Total CO_2 formed
Isolated leaf	1.0 cc.	0.5 cc.	0.5 cc.	2.0 cc.
Temperature in greenhouse during experiment	23–25°C	25–28–21°C	21°C	

The volume output of CO_2 of isolated leaf on the 1st day after isolation was rather small, compared with that in the attached (control) leaf. After the experiment, the leaf was removed from the leaf chamber and put on the moist soil until the next morning, when the following experiment was begun. Just before its beginning, the isolated leaf was again enclosed within the leaf chamber, and then the measurement of CO_2 output from it was done. The results, obtained on the 2nd, 3rd and 4th day after isolation, were as follows:

TABLE V.

	Total CO_2 formed (8 a.m.–11 p.m.)	Temperature in greenhouse during experiment
Control	28.5 cc.	20–28°C
Isolated leaf	on 2nd day after isolation 5.0 cc.	22–28°C
	on 3rd day after isolation 6.5 cc.	20–28°C
	on 4th day after isolation 7.0 cc.	20–28°C
	5th day after isolation, roots appeared at the notches of isolated leaf.	

The volume of CO_2 output from the leaf falls suddenly after isolation.

In the next experiment, other healthy, vigorous, and about one year old plants were taken as material. The plants were submerged under a warm bath of about 35°C for 8 hours, from 2 a.m. to 10 a.m.

The volume output of CO_2 from the leaf after warm bath treatment was measured. Immediately after that the treated leaf, which was still attached to the middle part of the stem of the treated plant, was closed within the leaf chamber, and determination of CO_2 output was made. The results are given in following table:

TABLE VI

	CO_2 formed (10 a.m.–1 p.m.)	CO_2 formed (1 p.m.–5 p.m.)	CO_2 formed (5 p.m.–10 p.m.)	Total CO_2 formed
Control	11.0 cc.	12.0 cc.	5.0 cc.	28.0 cc.
Treated leaf	6.5 cc.	2.5 cc.	5.5 cc.	14.5 cc.
Temperature in greenhouse during experiment.	25–30°C	30–22°C	22°C	

The volume of CO_2 formed on the first day after the warm bath treatment was smaller than that of the control. And it is remarkable that the volume output of CO_2 by the treated leaf during 3 hours immediately after treatment was 6.5 cc., but that it decreased to 2.5 cc. during the next 5 hours, and then increased again. It is very interesting if this phenomenon

be considered together with the variation in acidity in the leaf after warm bath treatment which was seen in the preceding experiment. By comparing these two phenomena to each other, the following fact will be apparent.

Immediately after warm bath, the acidity in the treated leaf suddenly decreases, during which period, however, the CO_2 production from the treated leaf is larger, and then the acidity in the treated leaf rises considerably, while on the contrary, the volume output of CO_2 from the treated leaf is very small. After this the acidity again decreases, while the volume of CO_2 emission from the treated leaf becomes larger. In other words, after warm bathing, when the acidity in the treated leaf decreases, the volume output of CO_2 increases, and *vice versa*. This fact proves that the variation in acidity in a treated leaf after warm bathing is connected with the respiration. In control material, such phenomena did not take place.

The total volume of CO_2 output from the treated leaf from 10 a.m. to 10 p.m. of the 1st, 2nd, 3rd and 4th day after warm bathing was as follows:

TABLE VII

	CO_2 formed (10 a.m.–10 p.m.)	Temperature in the greenhouse during experiment
Control	28.0 cc.	22–28°C
Treated leaf	1st day after warm bathing 14.5 cc.	22–30°C
	2nd day after warm bathing 2.5 cc.	20–30°C
	3rd day after warm bathing 4.5 cc.	22–30°C
	4th day after warm bathing 6.0 cc.	20–30°C

The volume of CO_2 emitted from the treated leaf was small, especially so on the 2nd day after warm bathing, compared with that of control.

Further experiment was made in which another healthy, vigorous, and about half a year old leaf which was still attached to the middle part of stem was closed within the leaf chamber and measurement of the volume output of CO_2 by this leaf was made (control experiment). After this experiment was finished, the air in the leaf chamber was replaced entirely by H_2-gas, i. e., the leaf was placed under anaerobic state and the measurement of CO_2 emission from the leaf was worked out. The results were as follows:

TABLE VIII

	CO_2 formed (8 a.m.–11 p.m.)	Temperature in the greenhouse during experiments.
Control	18.1 cc.	20–30°C
Treated leaf	4.8 cc.	20–30°C

The volume output of CO_2 from the leaf, which was placed under anaerobic state, decreased to about ¼ that of control. After the leaf was placed under this anaerobic condition for 48 hours, it was returned to normal aerobic condition. Measurement of the volume output of CO_2 after treatment was also made. The volume of CO_2 produced by a leaf immediately after treatment was as follows:

TABLE IX

	CO_2 formed (8 a.m.–1 p.m.)	CO_2 formed (1 p.m.–6 p.m.)	CO_2 formed (6 p.m.–11 p.m.)	Total CO_2 formed
Control	7.5 cc.	8.5 cc.	2.1 cc.	18.1 cc.
Treated leaf	2.5 cc.	0.5 cc.	1.5 cc.	4.5 cc.
The temperature in the greenhouse during experiment	23–25°C	25–30–20°C	20°C	

The leaf, which was returned from the anaerobic to the aerobic state, produced 2.5 cc. CO_2 during the first 5 hours, and 0.5 cc. during the next 5 hours, and then 1.5 cc. If one considers this fact of variation in the volume output of CO_2 from the operated leaf, as compared with the change in acidity after treatment, detail of which was described in preceding pages, the following interesting facts are apparent, as was seen in the case of warm bath treatment:

The acidity in the treated leaf decreased during the first 4 hours immediately after the treatment; on the contrary, the production of CO_2 from the treated leaf during this period was large. During the next 4 hours, the acidity in the treated leaf suddenly rose, but the volume of CO_2 from the treated leaf suddenly fell during this time. Then the acidity in the treated leaf again decreased, while the volume of CO_2 emitted by the treated leaf again increased. These facts will confirm the explanation which was given

above for the variation in the acidity after introducing anaerobic respiration. The same phenomenon was also seen in the case of warm bath treatment, as already described. On the 1st, 2nd, 3rd and 4th days after placing in H_2-gas, the total volume of CO_2 output from the treated leaf was as follows:

TABLE X

	Total CO_2 formed (8 a.m.–11 p.m.)	Temperature in the greenhouse during experiments
Control	18.1 cc.	20–30°C
Treated leaf	on 1st day after treatment 4.5 cc.	20–30°C
	on 2nd day after treatment 2.5 cc.	20–30°C
	on 3rd day after treatment 4.5 cc.	20–30°C
	on 4th day after treatment 6.8 cc.	20–30°C

The volume of CO_2 produced by the treated leaf was smaller than that of control, especially so in the second day after treatment. This fact was already seen in the case of warm bath treatment.

Miscellaneous experiments, in which the measurements of volume of CO_2 output from the variously treated *Bryophyllum* plants were carried out will be described hereunder. The writer placed a healthy and vigorous plant in the cold glasshouse, in which other environmental conditions were almost equal to those of the normal greenhouse, except only that the temperature was lower. The plant was placed in the cold glasshouse at the temperature of about 10°C for 3 weeks, and then the volume of CO_2 output from this plant was measured. The leaf, attached to the middle part of the stem of this plant, was closed within the leaf chamber, and the measurement made. As control, the same aged leaf, attached to the middle part of the stem of another plant which was placed in the normal greenhouse (20–30°C), was used. The results are given in following table:

TABLE XI

	CO_2 formed (10 a.m.–5 p.m.)	Temperature in the greenhouse during experiments
Control	16.0 cc.	25–28°C
Treated leaf	2.5 cc.	10–20°C

In another plant in the greenhouse (20–30°C) stem was girdled, and the determination of volume of CO_2 emission from the leaf, attached to the upper part of the operated portion in stem, was carried out. As control, a leaf attached to the corresponding node in the stem of another plant about of equal age which was not operated was used.

TABLE XII

	CO_2 formed (10 a.m.–5 p.m.)	Temperature in the greenhouse during experiments
Control	11.0 cc.	20–30°C
Treated leaf	2.0 cc.	20–30°C

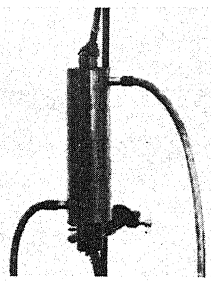


Fig. 24.

Another experiment was carried out in which a stem cutting was used as material. About one year old plants were selected out. As a control experiment, the middle part of the stem of such a plant was used. This part was closed within a hollow cylinder of nickel-plated copper (see Fig. 24), without being taken away from the plant. By using this hollow cylinder, the measurement of volume of CO_2 output from the stem was made. The methods, used for estimation of CO_2, were the same as in the case of the experiment in which CO_2 volume from the leaf was measured. The result was as follows:

TABLE XIII

	CO_2 formed (10 a.m.–5 p.m.)	Temperature in the greenhouse during experiments
Control	6.0 cc.	about 27°C

After this control experiment was finished the same part of the stem was detached. By the use of this detached piece the volume of CO_2 emitted was measured. During this time, the stem cutting was connected at the basal end with a rubber tube filled with water at room temperature, in order to avoid drying out of the stem. The result thus obtained was as follows:

TABLE XIV

	CO_2 formed (10 a.m.–5 p.m.)	Temperature in the greenhouse during experiments
Stem cutting	1.5 cc.	about 27°C

That is to say, the volume of CO_2 emitted from the stem cutting is smaller than when it forms the integral part of a plant. Detailed investigations on regeneration in stem cutting in various plants will be carried out by the writer in the near future.

From the results of the experiments described above, it may be said that in the case of isolation, warm bath treatment, the introduction of anaerobic respiration and other experimental treatments led to the decrease of the volume of CO_2 output during the oxygen respiration.

The Respiration Quotient in the Attached and Isolated Leaf of *Bryophyllum calycinum*

In this experiment, the CO_2/O_2 value in the respiration of attached and isolated leaf was measured. For the estimation of the volume of CO_2 and O_2 in this experiment, HEMPEL's apparatus was employed. The volume of O_2 was determined by the adsorption of alkaline solution of pyrogallol, and that of CO_2 by the adsorption of the solution of KOH. Healthy, vigorous, about one year old plants were selected out for the material. The potted plant material was placed in the thermostat of 28°C. One leaf, attached to the middle level of this material, was closed within the leaf chamber, which was used in the preceding experiment (see p. 238). In this experiment, however, the air in the leaf chamber was not replaced by fresh one during the experiment. After the leaf to be used was closed within the leaf chamber, the outer blind door of the thermostat was shut. Hence, the whole plant body was placed in a dark place. In this state, the material was left for 24 hours. Then, gas analysis was carried out by using the air which has been closed for 24 hours in the leaf chamber. 100 cc. air, which were taken from the leaf chamber, were used for each determination. The result was as follows:

TABLE XV

O_2 decrease during 24 hours	CO_2 increase during 24 hours	$\frac{CO_2}{O_2}$
8.8 cc. 28°C and 1 atm.p.	4.3 cc. 28°C and 1 atm.p.	0.5

The CO_2/O_2 value in the respiration of attached leaf of *Bryophyllum calycinum* in the dark is 0.5. This result served as the control. Next, other similarly aged plants were selected out, as used in the above control experiment. A leaf, attached to the middle level of the stem, was detached from the stem, and closed within the leaf chamber. Then this isolated leaf was placed in the thermostat at 28°C. After doing so, the outer blind door of the thermostat was shut. Thus the isolated leaf was placed in the dark place, as was done in the case of the attached leaf. After 24 hours, the determination was made by using 100 cc. of air from the leaf chamber. The results are given in the following table.

TABLE XVI

	O_2 decrease during 24 hours	CO_2 increase during 24 hours	$\frac{CO_2}{O_2}$
Control	8.8 cc.	4.3 cc.	0.5
Isolated leaf on the 1st day after isolation	5.1 cc.	0	
Isolated leaf on the 3rd day after isolation	3.0 cc. 20°C and 1 atm.p.	1.0 cc. 20°C and 1 atm.p.	0.3

CO_2 increase on the 1st day after isolation in the gas of leaf chamber, in which the isolated leaf was closed, could not be determined by this method, while O_2 decrease in the same material was determined to be 5.1 cc. This amount of O_2 might be available for formation of organic acid in the leaf. The CO_2/O_2 value on the third day after isolation was 0.3. These facts may indicate that the oxygen respiration in the isolated leaf has been depressed.

Quantitive Determination of Acetaldehyde and Ethyl Alcohol which are formed in the *Bryophyllum* Leaf

As has been seen in the preceding pages, the products of abnormal metabolism, especially the products of intramolecular respiration, are con-

nected with the occurrence of regeneration in *Bryophyllum calycinum*. Owing to intramolecular respiration, various substances are formed in the plant body, among which acetaldehyde known as the precursor of ethyl alcohol in the intramolecular respiration has a special physiological meaning. For instance, the experimental results of NEUBERG (1918), NEUBERG and GOTTSCHALK (1924), BORESCH (1926 and 1928), and NEITHAMMER (1928) and others show this fact.

In the following experiments, therefore, the writer intended to determine acetaldehyde and ethyl alcohol which are produced in the leaf of *Bryophyllum calycinum* in various cases. The method of determination of acetaldehyde in this experiment was done after NEUBERG and GOTTSCHALK (1927), as follows: When acetaldehyde reacts with hydroxylamine sulfate, free H_2SO_4 and oxim are produced. This free H_2SO_4 is titrated with NaOH. In the present experiments, 10 cc. of sample were taken and mixed with 0.2 cc. of 4% hydroxylamine sulfate. This mixture was kept at 30°C for 1 hour, and titrated with 0.1 N NaOH by micro-burette, using methyl orange as indicator. Basing upon the reading of 0.1 N NaOH in micro-burette, the milligram of acetaldehyde was calculated. The determination of ethyl-alcohol was made according to NICLOUX's method. Ethyl alcohol solutions of various concentrations, i. e. from 0.01 to 2.00% were prepared. 5 cc. of each solution were put in the test tubes and 1 cc. of 2% potassium bichromate and 5 cc. of concentrated H_2SO_4 added to it. This series shows difference in colour. By employing this series as the standard, the estimation of ethyl alcohol was made. In these experiments, 5 cc. of sample to be tested were put in the test tube, and mixed with potassium bichromate and H_2SO_4 as being done in the standard series. The colour, which this mixture produced, was compared with that of the standard series. In this way, the volume percentage of ethyl alcohol in sample was determined. In the experiment, 100 g. of fresh leaves were used in each determination. They were torn in small pieces which were distilled with boiling vapour, cooling with LIEBIG's condenser of 1 m. length, which was connected with the spiral cooler with about 1 m. coil length. This spiral cooler were cooled by ice water.(1) Exactly 100 cc. of the distillate, thus obtained, was taken for determination. The experiments in this series were worked out in five days under the temperature of 25°C. All determinations were made at 2 o'clock in the afternoon throughout the experiments.

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(1) See BORESCH (Biochem. Z., 1928).

1. Quantity of Aldehyde and Alcohol which are formed in the Leaves attached to the Upper and Middle Parts of the Stem

In the very young leaves which were growing at the top of the plants, and in adult, healthy, vigorous and about half a year old leaves which are attached to the middle part of the stem, the quantity of aldehyde and alcohol was as follows:

TABLE XVII

	Aldehyde	Alcohol
Very young leaves	8.5 mg.	0.11%
Adult leaves	1.7 mg.	0.04%

The results show that the quantity of aldehyde and alcohol is greater in growing young leaves than in adult ones. This experiment was carried out in May 1929.

2. Quantity of Aldehyde and Alcohol in the Isolated Leaves

500 g. of fresh weight of leaves, which are attached to the middle part of the stem and were about half a year old, were detached from the stem. These leaves were divided into 5 equal groups, each having 100 g. leaves. One of the groups (100 g.) was estimated for aldehyde and alcohol immediately after isolation. This served as control. The remaining four groups remained on moist soil under temperature of 18–25°C. The four groups were determined respectively at intervals of 24 hours for the successive 4 days after isolation. The results are collected in the following table:

TABLE XVIII

	Control	After 24 hours	After 2 days	After 3 days	After 4 days
Aldehyde	1.5 mg.	6.0 mg.	4.3 mg.	3.0 mg.	3.2 mg.
Alcohol	0.04%	0.09%	0.08%	0.07%	0.077%

In isolated leaves 4 times the weight of aldehyde and about twice the volume of alcohol were formed during 24 hours after isolation in comparison with the control, and then both decreased.

Qualitative analysis of aldehyde was also carried out in the following ways, with the distillate, which was obtained from the attached (control) and isolated leaves.

TABLE XIX

	SCHIFF's reaction	Anilin water	NESSLER's reaction
Control	−	−	+
Isolated leaf	+	+	+

3. Quantity of Aldehyde and Alcohol in the Leaves which were Subjected to Anaerobic respiration

About one year old potted plants were enclosed within a bell jar, which was filled with H_2-gas with a constant stream of H_2-gas passing. The plants were placed under this state for 48 hours at the temperature of about 25°C. After this treatment, the plant materials were returned to the normal aerobic condition, and then determination of aldehyde and alcohol was carried out. The leaves, attached to the middle part of the stem, were chosen out for this purpose.

TABLE XX

	Control	After 24 hours	After 2 days	After 3 days	After 4 days
Aldehyde	1.7 mg.	10.9 mg.	9.0 mg.	5.0 mg.	2.4 mg.
Alcohol	0.04%	0.095%	0.08%	0.08%	0.01%

The quantity of aldehyde in the first 2 days after treatment is rather great, and after that period it decreases as the days advance. The volume of alcohol in the treated leaves is large for 3 days after treatment, while on 4th day after treatment it rapidly decreases.

4. Warm Bath Treatment

About one year old plants were submerged in the warm bath at about 35°C. for 8 hours. Then they were returned to normal conditions. After a certain number of days, estimation of aldehyde and alcohol was carried

out by using the leaves which were attached to the middle level of the treated plants. The results were as follows:

TABLE XXI

	Control	After 24 hours	After 4 days
Aldehyde	1.7 mg.	5.0 mg	3.2 mg.
Alcohol	0.04%	0.14%	0.07%

A considerable amount of aldehyde and alcohol is formed in the leaves after warm bath treatment. This experiment was made from 10 to 14 May 1929. By isolation, by introducing anaerobic respiration, and by warm bath treatment, relatively considerable amounts of aldehyde and alcohol are accumulated in the leaves of *Bryophyllum calycinum*.

Discussion

The cause of regeneration in *Bryophyllum calycinum* was studied by many authors. GOEBEL (1908) maintained, from his experimental results of cutting off a part of mid-vein in the leaf, removing the top of plant and other similar treatments (using *Bryophyllum crenatum* mainly), that a correlation exists between the growth of growing point and bud formation on the attached leaf. So, according to GOEBEL, if the growth of growing point is depressed or arrested, the bud formation on the leaf should take place. CHILD and BELLAMY (1920) pointed out the inhibiting action of growing top upon the growth of bud on leaf of *Bryophyllum calycinum* and also the fact that by blocking this action the regeneration on the leaf is caused. REED (1923) applied the hypothesis of the formative stuffs, which had been proposed by GOEBEL (1901), to regeneration in *Bryophyllum calycinum*. LOEB (1920 and 1924), judging from his quantitative experiments, came concerning the cause of regeneration to the conclusion that the accumulation of the sap in the plant body is the main factor to cause regeneration in *Bryophyllum calycinum*. OSSENBECK (1927) recognized the correlation between growing point and formation of shoots and roots on the leaf, as well as the specific substance which is concerned with this correlation.

Returning to the present writer's experiments described in the preceding pages, which consist in introducing the intramolecular respiration in the plant body, in treating by warm bath or in placing the whole plant in H_2-gas,

the writer succeeded in producing roots and shoots on the leaves, which are still attached to the stem, and also adventitious roots on the stem. Injection with the chemical substances, which are assumed to be the intermediate and end products of intramolecular respiration, brought about similar results. Especially, ethyl alcohol is effective to produce roots and shoots on attached leaves. From these facts, it may be said that the products, which result from the intramolecular respiration, are concerned in the occurrence of regeneration in an attached leaf of *Bryophyllum calycinum*. The experimental results of the examination of the acid metabolism, CO_2 production, and accumulation of substances in leaf after isolation and after introducing anaerobic respiration showed that the diurnal changes of acidity and CO_2 output, and accumulation of metabolic products etc. in leaves after isolation are exactly similar to those which occur after introducing anaerobic respiration. From these facts, the writer supposes that by isolation incomplete respiration is induced in the leaf, and the products, which result from such metabolism, stimulate the occurrence of regeneration in isolated leaves of *Bryophyllum calycinum*. The incompleteness of respiration in the isolated leaf of this plant will also be proved from the results of experiments which consist in estimating the CO_2/O_2 value in the isolated and attached (control) leaves. The writer believes that the cause of occurrence of regeneration is connected with the catabolic processes, especially with intramolecular respiration, or other similar phenomena. That is, when we induce the abnormal metabolism in the leaf or in stem by isolation or other treatments, the regeneration in leaf or stem should take place. Abnormal metabolism in this case may be similar to intramolecular respiration. The products in this kind of respiration stimulate the occurrence of regeneration in leaf or stem.

Conclusion and Summary

1. When abnormal catabolism is brought about in *Bryophyllum calycinum* by means of warm bath, keeping within H_2-gas and other treatments, the production of shoots and roots at the notches of leaf still attached to the stem (in some cases the production of adventitious roots on stem) is induced. It seems that this abnormal catabolism is similar to intramolecular respiration.

2. By isolation of the leaf of this plant from the stem, the similar abnormal metabolism is induced as is seen in the case of experimental treatments.

3. Occurrence of regeneration in both the attached and the isolated

leaf of this plant seems to be connected with the products of intramolecular respiration.

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Abstracts Nos. 90-179

(Referring to the principal papers on Botany and related subjects which have appeared in Japan chiefly during January–June 1930).

90. On the Influence of Copper Sulphate on the Growth of *Piricularia Oryzae*, with Special Reference to the Temperature as an Environmental Factor. (Japanese with English résumé). Takuji ABE. (Ann. Phytopath. Soc. Japan 2, 1930, 171–196, 10 figs.).

The mycelium of *Piricularia Oryzae* cultivated on potato agar decoction containing 1% sucrose grows most vigorously at 28°C. Under this temperature the mycelial growth is stimulated by the addition of $CuSO_4$ between 1/400 and 1/6000 mol., and most vigorously at 1/1000 mol.

The conidial formation is retarded by the high temperature (most remarkable at ±36°) as well as by $CuSO_4$ at higher concentration than 1/6000 mol., no conidial formation taking place at 1/400 mol. When the concentration of $CuSO_4$ is higher than 1/400 the chlamydospores are produced.

91. Ueber den Ursprung des dreigliederigen Quirls von *Gardenia jasminoides*, ELLIS. Toichi ASAI. (Japan. Jour. Bot. 5 1930, 27–34, 3 Textabb.).

92. Ueber das Vorkommen und die physiologische Bedeutung des Daphnins bei *Daphne odora*. Toichi ASAI. (Acta Phytochimica 5, 1930, 9–21, m. 5 Abb.).

Der Verfasser hat das schon vor geraumer Zeit in *Daphne mezereum* entdeckte Glukosid Daphnin in einem Zierstrauch, *Daphne odora* THUNBERG, wieder aufgefunden. Der Befund wurde durch die Hydrolyse der Substanz in Daphnetin und Glucose festgestellt. Der Daphningehalt verschiedener Pflanzenteile, insbesondere der Blüten und der Laubblätter, wurde in verschiedenen Jahreszeiten quantitativ untersucht. Der grösste Gehalt (21.78% der Trockensubstanz) an Daphnin wurde in den sich entfaltenden Knospen konstatiert, während die abfallenden Laubblätter noch 2.94% davon enthielten. Aus bevorzugtem Vorkommen des Daphnins in der Epidermis der oberirdischen Organe sowie aus dem Lichtabsorptionsverhältnis der Substanz hat der Verfasser geschlossen, dass Daphnin, wie Flavonglykoside, als das Schutzmittel gegen schädliche ultraviolette Sonnenstrahlen dient. Eine ähnliche Betrachtung wurde auch über das Vorkommen des Cumarins (als Orthocumarsäure) in verschiedenen Pflanzen aufgestellt. Autor.

93. Aster Yellows in Japan. (Japanese). Teikichi FUKUSHI. (Agric. & Hortic. 5, 1930, 577–584, 3 figs.).

It is scarcely possible to find by any means the causal organism of this disease, and consequently the author considers it to be a virus disease.

94. On the Mosaic Disease of Tobacco. (Japanese). Teikichi FUKUSHI. (Jour. Plant Prot. 16, 1929, 217–232, 269–276, 333–339, 385–392).

A detailed account of the mosaic disease of tobacco which has been known in Japan since 1857. The literature is fully summarized; the symptoms, pathological anatomy,

host range, and the nature of the virus are described, and the hypotheses concerning the cause of the tobacco mosaic are critically reviewed. Author.

95. On the Leaf-spot Fungus of Lettuce. (Japanese). Teikichi FUKUSHI. (Jour. Plant Prot. **16**, 1929, 449–452).

The causal fungus of this disease is *Cercospora longissima* TRAV. (=*C. lactucae* STEVENS=*C. lactucae* WELLES).

96. Effects of Certain Alkaloids, Glucosides and Other Substances upon the Infectivity of the Mosaic Tobacco Juice. (With Japanese résumé). Teikichi FUKUSHI. (Trans. Sapporo Nat. Hist. Soc. **11**, 1930, 59–69).

The author has subjected the juice of mosaic tobacco to the action of various alkaloids, glucosides and ethereal oils, and found that it is very resistant against them. To cite only few instances, 2% oil of mustard and 5% digitalin were able to destroy the virulence of mosaic juice, only after 5 days of continual action.

97. Nature and Significance of Structural Chromosome Alterations Induced by X-rays and Radium. Thomas Harper GOODSPEED and Priscilla AVERY. (Cytologia **1**, 1930, 308–326, 3 pls. and text-figs.).

In the meiosis of second and third generation progenies derived after the treatment of *Nicotiana tabacum* with X-rays and radium the authors could observe the following types of chromosome reorganization, viz. attachment, translocation, deletion, fragmentation, altered valency. Plants which have undergone such types of chromosome alteration are viable to relatively high degree, though in *Drosophila*, etc. such alterations might often lead to lethality. Further, such chromosome reorganization may give rise to stable derivatives often distinct in external morphology from the original race.

98. Studies on *Polystictus sanguinea*. (Japanese with English résumé). Shigekatsu HIRAYAMA. (Bull. Sc. Friends' Assoc. Higher School Agric. & Forest. Mie **1**, 1929, 21–42, 1 pl. and 2 text-figs.).

Polystictus sanguinea (L.) FR., of which, as the author thinks, *P. cinnabarinus* (JACQ.) FR. is merely a synonym, can be grown easily on artificial media. The mycelial growth is much better on agar than on liquid media, and on some solid ones the hymenia or the sporophores are developed with the production of numerous spores. The optimum growth takes at 40°C. The red pigment contained in the mycelial cells of this fungus shows against various reagents nearly the same reactions as the "xanthotrametin" from *Trametes cinnabarina* (ZOPF), and light seems at least to promote its production. As shown by the inoculation experiments, the fungus seems to be omnivorous, and is destructive much more towards dicotyledonous than coniferous woods.

99. On the Black Spot Disease of *Dioscorea alata* and *D. Batatas*. (Japanese with English résumé). Kazuo GOTOH. (Jour. Soc. Trop. Agric. **1**, 1929, 301–313).

The disease is due to *Gloesporium pestis* MASSEE, provided that the presence or absence of setae may not be the important character for classification, as believed by many investigators. The pathogenicity of the fungus was confirmed experimentally.

100. Karyologische Studien über die Gattungen *Trillium* und *Paris*. (Japanisch). Kazuo GOTOH und Isamu STOW. (Japan. Jour. Gen. **5**, 1930, 114–117).

Nach den bisherigen Studien verschiedener Forscher an den *Trillium*- und *Paris*-arten beträgt die Chromosomenzahl $x=6$ oder 12 und $2x=12$ oder 24, d. h. die Grundzahl soll immer 6 sein. Nach den neueren Studien der Verf. beträgt die Grundzahl der Chromosomen bei den Pollenmutterzellen 5, während die somatische Zahl 10 (=5 II), 20 oder 15 (=5 III) beobachtet wurde. Die Ursache der Abweichung zwischen den bisherigen und neuen Studien bezüglich der Chromosomenzahl ist nicht ganz klar, doch machen die Verf. darauf aufmerksam, dass bei karyologischen Studien das Zerschneiden einiger Chromosomen, besonders der langen, entweder natürliche oder künstliche (z. B. beim Schnittmachen) nicht unmöglich sein dürfte. Weiter, unter 5 Arten Chromosomen von beiden Gattungen sind 4 in Gestalt gleich; einer von denselben ist doch bei verschiedenen Arten verschieden in Gestalt. Es ist immer stäbchenförmig und trägt ein oder zwei winzige Körperchen, von denen ein dem Trabant oder der Satellite ähnlich ist. Ein dieser Körperchen trägt einen hornartigen Fortsatz, welcher entweder lang oder kurz sein mag. Die Ansicht der Verf. über die Herkunft aller solchen Formen wird erwähnt.

101. Biologic Forms of *Albugo candida* (Pers.) Kuntze on Some Cruciferous Plants. Makoto Hiura. (Japan. Jour. Bot. 5, 1930, 1–20).

102. On *Melampsorella elatina* in Japan. (Japanese). Naohide Hiratsuka. (Agric. & Hortic. 5, 1930, 153–164, 2 text-figs.).

The fungus which is the cause of the witches' broom in *Abies* must be rightly called *Melampsorella elatina* (Alb. et Schw.) Arth. In Japan its aecidium generation is found parasitic on several species of *Abies*, as, for instance *A. firma, homolepis, Veitchii*, etc., while its uredo- and teleutospore generation are found on certain species of *Cerastium* and *Stellaria*. The author was able to infect artificially *Cerastium vulgatum* var. *glandulifera* by means of aecidiospores got on *A. Mayriana*.

103. *Pucciniastrum* of Japan. (Notes on the Melampsoraceae of Japan III). Naohide Hiratsuka. (Bot. Mag. Tôkyô 44, 1930, 261–284).

An enumeration of 19 species of *Pucciniastrum* in Japan. 1 new species, *P. Fagi* Yamada wird beschrieben. The paper ends with the index of fungi and of the hosts.

104. Nuntia ad Floram Japonicae VI. (With Japanese résumé). Masaji Honda. (Bot. Mag. Tôkyô 44, 1930, 316–318).

5 plants are enumerated, of which 2 are new species and 1 a new variety, viz. *Cacalia zigzag* sp. nov., *Geranium kai-montanum* sp. nov., *Cardiandra alternifolia* Sieb. et Zucc. var. *oppositifolia* var. nov.

105. On the Mode of Primary Infection through Sclerotia and Field Observations on the Basidiospore-formation in *Hypochnus Sasakii* Shirai of Rice-plants. (Japanese). Suehiko Ikata and Takesi Hitomi. (Jour. Plant Prot. 17, 1930, 12 pp., 1 pl.).

The sclerotia of *Hypochnus Sasakii* on rice plants fall almost wholly on the field before the harvest, so that their great number is found in the field; for instance, the authors have counted in average 18.3 sclerotia per 30 cm^2 field surface. Such sclerotia hibernate there and cause the infection of rice-plants in the next year. The formation of basidiospores in this fungus which is parasitic on rice seems never to have been observed

heretofore. The authors were able to observe their abundant formation at night in leaves and leaf-sheaths during August–September.

106. Chromosome Number in *Dianthus*. I. T. Ishii. (Cytologia **1**, 1930, 335–339, 24 figs.).

The author, by studying the chromosome number in root-tips of 24 species of *Dianthus* has found that among them 10 species possess 30, 2 60, and 12 90 chromosomes respectively. The author has further observed a certain relation between the chromosome number of different species on one hand and the size or weight of their seeds on the other. Thus, for instance, in the species with 30 and 90 chromosomes seeds belong to the small and the large group respectively, while in those with 60 chromosomes they may belong to any of large, small or medium groups.

107. Symbolae ad Mycologiam Japonicam. IV–V. Tokutaro Ito. (Bot. Mag. Tôkyô 44, 1930, 89–93, 151–157).

An enumeration of the Japanese species of *Asterostromella* (2 spp.) and *Hymenochaete* (11 spp.).

108. On the Serological Classification of the Root-nodule Bacteria of Leguminous Plants. (With Japanese résumé). Tadao Jimbo. (Bot. Mag. Tôkyô **44**, 1930, 158–168).

The serological studies on the root-nodule bacteria of *Wistaria floribunda*, *W. brachybotrys*, *W. venusta* and *Robinia pseudoacacia* were performed to know their systematic affinity. 21 pure cultures were employed. The results were firstly, that the bacteria which are serologically different may be observed in different individuals of one and the same species, secondly that, on the contrary, those which are serologically identical may be found in different species, and thirdly that those of serologically different sorts may be found in different nodules of one and the same individual.

109. On the Life-history of *Uredinopsis pteriditis*, with a Special Bearing on its Peridermal Stage. (Japanese with English résumé). Senji Kamei. (Ann. Phytopath. Soc. Japan **2**, 1923, 207–228, 1 pl.).

According to Weir and Hubert the teleutospores of *Uredinopsis pteriditis* seem to germinate without hibernating and to infect the needles of *Abies* in late summer or fall, so that the aecidia appear early in the next spring. The author has found, however, that the teleutospores of this fungus found in Sapporo germinate first in spring after overwintering and infect the young needles of *Abies Mayriana*. The spermogonia and peridermia formed on the latter by the inoculation of the sporidia of *Uredinopsis pteriditis* are described in detail.

110. Cytological Studies of Pollen Mother-cells of *Rhoeo discolor*, Hance with Special Reference to the Question of the Mode of Syndesis. Kazuo Katô. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B **5**, 1930, 139–161, 2 pls. and 9 text-figs.).

In the heterotype division of the pollen mother-cells in *Rhoeo discolor* the nuclear thread is represented by a ring or chain composed of 12 univalent chromosomes connected end-to-end by thin portions, while the formation of gemini is not observed. In 4 out of these 12 univalents the spindle fibre attachment is subterminal (J-shaped or heterobrachial chromosome), and in the remaining 8 median (V-shaped or isobrachial chromosome). The

4 heterobrachial chromosomes occupy the diagonally opposite positions to each other in each ring, and on each side of the latter 4 isobrachial chromosomes are inserted between each two sets of heterobrachial ones. The latter may be conjoined either at their proximal (i. e. near to the spindle fibre attachment) or distal ends; they are distributed to the same pole in the former case and to different poles in the latter.

From all his observations the author concludes as follows: the mode of syndesis in this plant is a sort of parasyndesis in which the homologous mates are prematurely separated from each other, but the segmentation of the spireme thread is postponed, and a chromosome ring is produced.

111. Chromosome Arrangement in the Meiotic Divisions in Pollen Mother-cells of *Rhoeo discolor*, HANCE. Kazuo KATÔ. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B 5, 1930, 229–238, 30 figs.)

In the heterotype metaphase in pollen mother-cells of *Rhoeo discolor* the chromosomes form a ring (cf. the preceding No.), but in the heterotype anaphase and the homotype metaphase they are free from each other. In normal cases 5 out of 6 chromosomes arrange themselves at the periphery with 1 at the centre most frequently. When the chromosome number is abnormally 5 or 7, the arrangement of 5 chromosomes at the periphery in the former case and that of 6 at the periphery and 1 at the centre in the latter are most frequent. All these arrangements resemble the stable configuration of MAYER's floating magnets. The arrangement of 6 chromosomes at the periphery and none at the centre in the heterotypic anaphase is very frequent, and this is due, as the author thinks, to the fact that the chromosomes which have formed a ring in the metaphase are freed from each other, only in the anaphase. Further, the fact that in the homotype division the arrangement of chromosomes resembles only to a small degree the configuration of floating magnets is because the long chromosomes in this plant have to move in a relatively confined space.

112. On the Affinity of the Cultivated Varieties of Rice Plants, *Oryza sativa* L. Shigemoto KATO, Hirosi KOSAKA, Shiroku HARA, Yoshio MARUYAMA and Yoshisuke TAKIGUCHI. (Jour. Dpt. Agric., Kyushu Imp. Univ. 2, 1930, 241–276).

The authors distinguish two types of rice varieties which they call *Japonica* and *Indica* respectively. The first ones are indigenous to Japan proper and Corea, while the second ones are the natives of Southern China, India, Java, etc. All varieties in both types are self-fertile, while the hybrids between the varieties of the two types are much less fertile. The latter fact is, as shown by cytological studies, due to the formation of many imperfect pollen grains in consequence of the abnormalities seen already in the initial stage of their development. The difference in the grade of sexual affinity between any two varieties within one and the same type and that between two varieties from the different types are clearly recognizable by studying the serodiagnostic reactions (''Ringprobe'' for the precipitin reaction with ''Absättigungsverfahren'').

113. Eine Tabelle der Chromosomenzahl bei den Leguminosen. (Japanisch). Jirô KAWAKAMI. (Bot. Mag. Tôkyô 44, 1930, 319–328, 72 Textabb.).

Die Chromosomenzahl (n oder $2n$) bei 35 zu 14 Gattungen gehörenden Leguminosenarten wird in der vorliegenden Mitteilung angegeben. Die haploide Zahl schwankt zwischen 6 und 24. Die Untersuchungsresultate anderer Forscher sind hinzugefügt.

Die aus den Verfs. Studien gezogenen allgemeinen Schlüsse sind wie folgt: 1. Die

Mehrzahl der Leguminosen enthalten die gerade Chromosomenzahl (z. B. 6, 8, 12, 24 usw.), wenn auch bei einigen Gattungen sie ungerade ist (z. B. 7, 9, 11); 2. bei einer und derselben Unterfamilie oder sogar bei einer und derselben Gattung ist die Chromosomenzahl bisweilen keineswegs einheitlich, z. B. bei Genisteae $n = 8$ oder 24, bei *Trifolium* 7x oder 8x; 3. bei einer und derselben Art ist sie oft verschieden, z. B. bei *Cassia mimosoides* und *Vicia unijuga* beträgt sie 8 oder 16 bzw. 6, 12 oder 18.

114. Genomanalyse bei *Triticum* und *Aegilops*. H. KIHARA. **I. Genomaffinitäten in tri-, tetra- und pentaploiden Weizenbastarden.** H. KIHARA und I. NISHIYAMA. (Cytologia 1, 1930, 263–284, 13 Textabb.).

In der Einleitung zitiert H. KIHARA zunächst die bisherigen experimentellen Studien verschiedener Forscher über die auto- und allopolyploiden Pflanzen, wobei die hauptsächlichen Unterschiede zwischen beiden hervorgehoben sind. Ob eine Pflanze auto- oder allopolyploid ist, kann mittelst den Bastardierungsversuchen entschieden werden, indem man z. B. durch die Kombination einer tetraploiden Pflanze mit einer bekannten diploiden einen triploiden Bastard herstellt und die Reduktionsteilung der Pollenmutterzellen bei dem letzteren untersucht. Wenn dabei die $3n$ Univalente aufgefunden werden können, kann man sofort auf die allopolyploide Natur der in Rede stehenden tetraploiden Pflanze schliessen; wenn dagegen dabei n Bivalente und n Univalente sich befinden, kann man nicht ohne weiteres weder für die allo- noch autopolyploide Natur der zur Untersuchung genommenen Pflanze entscheiden. Im Falle der Allopolyploide muss man weiter das Mass der Affinitäten einzelner Glieder von zwei oder mehreren sie zusammensetzenden verschiedenartigen Genomen studieren, welches am besten durch die Zahl der zwischen ihnen ausgebildeten Bindungen zu Bi- oder Trivalenten angezeigt werden kann.

Folgt die Zusammenarbeit von KIHARA und NISHIYAMA. Bis jetzt hat man innerhalb der Gattung *Triticum* drei verschiedene Genome zu je 7 Chromosomen festgestellt, welche durch die Buchstaben A, B und D unterschieden werden. Die Verf. haben nun, wie oben in der Einleitung erörtert, die Reduktionsteilung der Pollenmutterzellen ben den triploiden, tetraploiden und pentaploiden Weizenbastarden untersucht. Nach den Resultaten solcher Untersuchungen kann man eine schwache Affinität zwischen den Gliedern aus A- und B-Genom nachweisen, indem in einigen Fällen ein Chromosom aus dem letzteren Genom zu den aus den Gliedern des ersteren zusammengesetzten Bivalenten hinzutreten kann, um damit einen Trivalent auszubilden. Ebenso kann man auf das Vorhandensein einer schwachen Affinität zwischen den Gliedern aus B- und D-Genom schliessen.

Einige allgemeinen auf den Grund der Untersuchungsresultaten der Verf. gezogenen Schlüsse über die Entstehung der polyploiden Weizenarten sind in ihren eigenen Worten wie folgt. "Die polyploiden Arten sind wahrscheinlich durch Kreuzungen zwischen diploiden ($2n = 14$) Ausgangsarten entstanden. Ursprünglich wird wohl noch eine starke Affinität zwischen den Genomen der ältesten Arten bestanden haben. Im Laufe des genotypischen Differenzierungsprozesses und der mit ihm parallel gehenden Veränderung der Genome müssen sich ihre gegenseitigen ursprünglich starken Affinitäten nach und nach mehr oder weniger verloren haben, so dass ihre Kombinationen in Bastardierungen Polyploidie herbeiführen mussten. Darauf, dass die Affinitätsbeziehungen zwischen den heute normalerweise nichthomologen Genomen früher einmal vorhanden waren, weisen unsere Studien hin."

115. Ueber "osmiophile Plättchen" BOWENS in pflanzlichen Zellen. Kogane KIYOHARA. (Cytologia 1, 1930, 328–334, m. Textabb.).

Die "osmiophilen Plättchen" sind die von BOWEN als "neue Zellelemente" in pflanzlichen Zellen angedeuteten Gebilde, deren Peripherie durch Osmiumsäureimprägnierung stark geschwärzt wird. Nach den Untersuchungen des Verfs. des vorliegenden Aufsatzes an verschiedenen Pflanzen, wie *Hydrilla verticillata, Elodea canadensis, Vicia faba, Pilea viridissima* und an der Kartoffelknolle ergab es sich, dass diese sog. osmiophile Plättchen keineswegs neue Zellelemente darstellen, sondern kein anderes als die jungen Plastiden sind. Weiter, auf verschiedenen Reaktionen gestützt, hat der Verf. es festgestellt, dass freie Lipoide sich in der Grundsubstanz der Plastiden homogen verteilt befinden.

116. Contributiones ad Cognitionen Florae Asiae Orientalis. Gen'iti KOIDZUMI. (Bot. Mag. Tôkyô 44, 1930, 93–112).

An enumeration of Japanese plant species from various families. New species from some genera (*Epimedium, Cimicifuga, Agrimonia, Rubus, Corydalis*) are described.

117. The Inheritance of Mosaic Flower-colour in a Race of *Celosia cristata* L. (Japanese with English résumé). Hitoshi KOJIMA. (Bot. Mag. Tôkyô 44, 1930, 328–351, with text-figs.).

Seeds got by self-pollination of invididuals of a race of *Celosia cristata* with inflorescences which are scarlet and yellow mosaic-coloured produce the progeny composed of self-scarlets, mosaics and self-yellows. The progeny from the self-scarlet part of the inflorescence contain much more self-scarlet plants than those from the yellow part, moreover there is no considerable difference in the rate of segregation between the progeny from both parts. Self-scarlet progeny are either homo- or heterozygous. The self-pollination of apparently self-yellow plants gives rise in most cases to self-scarlets and mosaics besides self-yellows, though their ratio is very variable. The author has however succeeded in getting a fixed self-yellow strain by means of repeated self-pollinations.

The crossing experiments between homozygous self-scarlets and self-yellows have been followed till F_3, and moreover the back-crosses were performed. On the basis of the results of such experiments the author came to regard the case under discussion as that of a Mendelian hybrid, where self-yellows + mosaics on one hand and self-scarlets on the other should be treated as an allelomorphic pair.

The results of the author's experiments are explained as follows. We have here to do with two genes **A** and **a**, of which the former refers to scarlet colour, and is dominant to **a** referring to yellow colour. **A** is quite stable while **a** being unstable the mutation **a**→**A** takes place frequently, though **A**→**a** never occurs. Homozygous scarlet = **AA**, heterozygous scarlet = **Aa** and self-yellow = **aa**. Since sometimes some parts of self-yellows undergo the modification into scarlets on account of the gene mutation **a**→**A**, the mosaic plants may be derived from self-yellows.

118. Studies in the Histogenesis of RÖNTGEN-tumour, which I call Neoplasma Produced by the Irradiation of RÖNTGEN-rays in Plant Bodies. I. On "Thread-like Bodies" Discovered in the RÖNTGEN-tumours of Root-tips of *Pisum sativum*. (Japanese). KOMURO-Hideo. (Jour. Pathol. Soc. Japan 19, 1930, 736–738).

In X-rays tumours in root-tips of *Pisum sativum* in their primary stage there are some cell-groups of extraordinary size which are placed in the periblem or outside the plerom. Within them the author has found some filiform bodies of various size which are basophilous. Their function is yet unknown.

119. Beziehung zwischen der Wassertemperatur und dem Wachstum der Reispflanzen. Erste Mitteilung. Mantarô KONDÔ und Tamotsu OKAMURA. (Ber. Ôhara Inst. landw. Forsch. 4, 1930, 395–411, 2 Tafeln und 6 Textfig.).

Betreffend eine Rasse des Sumpfreises haben die Verf. die Beziehung zwischen dem Wachstum und der Temperatur des Feldwassers studiert. Danach beträgt für die Bestockung das Optimum ca. 34°C, für das Längenwachstum 30–32°, für das Gesamtpflanzengewicht 34°, für den Gesamtkörnerertrag 30°. Von 30° bis 39° erfolgen, je höher die Wassertemperatur ist, das Austreten der Rispe, das Blühen und die Reife umso später. Je 20° Zunahme der Temperatur verursacht ca. 4–8 Tage Verspätung des Rispenaustretens. Das Maximum liegt zwischen 39°–43.5°.

120. Carpobiological Studies of *Crinum asiaticum* L. var. *japonicum* BAKER. Takuji KOSHIMIZU. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B 5, 1930, 183–227, 2 pls. and 42 text-figs.).

The seed of *Crinum* is a soft fleshy mass made up of the endosperm with the embryo therein, protected outwards by the corky layer, evidently against dessication and infection. The specific gravity of fresh seeds is always less than 1, so that they can float even upon well-water. The author has put seeds in sea-water and solutions containing NaCl. The following is the result of the experiment in sea-water. Seeds float upon sea-water and germinate after more than 70 days. When a few leaves of the plumule which have there developed begin to be injured after certain days there is formed a separation layer in the upper part of leaf-sheath which prevents the extension of the injury into the sheath-base, the young seedling was observed to remain safe thus during more than two years. Seeds brought to a sandy place and germinating there are hardly able to bury there by themselves.

Lastly the author makes a discussion on the geographical distribution of the species of *Crinum*.

121. Labiatarum sino-japonicarum Prodromus. Eine kritische Besprechung der Labiaten Ostasiens. Yushun KUDO. (Mem. Fac. Sc. and Agric., Taihoku Imp. Univ. 2, 1929, 37–332).

Eine eingehende vergleichende Studie der japanischen und chinesischen Labiaten, welche auf die aus den verschiedensten Quellen Japans, Europas und Nordamerikas stammenden Herbarmaterialien gegründet ist, hat den Verf. veranlasst, die vorliegende Monographie zu bearbeiten.

Darin sind alle aus der in Rede stehenden Region bekannten Labiaten enthalten, und dabei sind eine Anzahl von Gattungen, Sektionen, Arten, Kombinationen neu aufgestellt. Allen diesen Formen wird eine ausführliche lateinische Diagnose angegeben. Die folgenden Gattungen sind neu: *Siphocranion, Hanceola, Clerodendranthus, Hensleia, Dielsia, Lamiophlomis, Meehaniopsis, Prunellopsis, Rubiteucris, Kinostemon, Rostrinucula.* Das Register der Pflanzennamen schliesst das Werk.

122. Studies on the Structure of Japanese Species of *Ranunculus*. Masao KUMAZAWA. (Jour. Fac. Sc., Imp. Univ. Tokyo, Sec. III (Bot.) 2, 1930, 297–343, 2 pls. and 18 text-figs.).

The chief points of interest elucidated by the author's anatomical and morphological studies on 16 Japanese *Ranunculus* species are as follows. Stem is either erect or

creeping in one and the same species, though both occur in *R. Kawakamii*. In the stem two types of leaf trace strands are distinguishable: in the one the strands run downwards from the petiole without fusing with any stem strand at least in the next internode, while in the other they join with the stem strand immediately after their entering the stem. Further in the seedlings each of three leaf trace strands belonging to a leaf forms the gap of its own (''trilacunar type''), though sometimes three strands leave only one common gap (''unilacunar type''). In some cases the stem bundles are surrounded entirely by the bundle sheath (''*Ranunculus* type''), while in others (as *R. sceleratus*) no such sheath occurs and we see only some fibrous mass on the phloem side (''*Anemone* type''). The former type occurs in the Monocotyledons and seems to be phylogenetically younger than the latter, hence the author considers *R. sceleratus* as one of the primitive representatives of the genus *Ranunculus*. The V-shaped xylem which is characteristic of the stem bundle of this family and also very often of that of the Monocotyledons is due to the reduction of the cambium and the formation of big cells in the lateral parts of the primary xylem.

In respect to other parts of interest in this paper cf. the original.

123. The Life-history and Physiology of *Synchytrium fulgens* SCHROET., with Special Reference to its Sexuality. Shunsuke KUSANO. (Japan. Jour. Bot. 5, 1930, 35–132, 19 text-figs.).

124. Cytology of *Synchytrium fulgens* SCHROET. Shunsuke KUSANO. (Jour. Coll. Agric., Imp. Univ. Tokyo **10**, 1930, 347–388, 3 pls. and 4 text-figs.).

The reproduction of *Synchytrium fulgens* is either sexual or asexual. In the latter case the gamete (asexual) enters the host-cell and grows there considerably to become the prosorus. The latter is furnished with one nucleus which is represented by a few granules at the periphery of a clear nuclear cavity. During the growing stage the nucleolus which newly appears discharges the chromatin globules which may pass from the nucleus into the surrounding cytoplasm; the irregular linin mass on the surface of the nucleus which the author calls ''crown'' serves for carrying such globules from the nucleus to cytoplasm. After the growing stage is over the nucleus of the prosorus (primary nucleus) undergoes the division. The spireme strings are then formed from extranucleolar elements, and the nucleolus begins again to discharge the chromatin globules. The spindle is formed and 5 chromosomes become visible at metaphase, though generally individual chromosomes are not discernible. After the formation of a certain number of secondary nuclei from the primary one the cytoplasm is divided into a number of multinucleate gametangia by means of a special cell-element ''karyodermatoplast,'' formerly discovered by the author in another fungus. In each gametangium nuclear divisions occur and the gamete nuclei are produced.

Each zygote resulting from the conjugation of the sexual gametes gives rise to a resting cell. The cytological behaviour during the growth of the resting cell incl. the mitosis of the primary nucleus, etc. is almost similar to what is observed during that in the prosorus.

125. The Meiotic Divisions in Pollen Mother-cells of the Sweet-pea (*Lathyrus odoratus* L.) with Special Reference to the Cytological Basis of Crossing-over. Takeshige MAEDA. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B 5, 1930, 89–123, 8 pls. and 7 text-figs.).

In the meiosis of pollen mother-cells of *Lathyrus odoratus* the parasyndesis occurs. The double nature of the spireme threads and the crossing between the two of such threads are seen from the late synizetic stage on. The spireme threads undergo the torsion. The author distinguishes three different types of crossing between them. From the view point of the cytological basis of crossing-over the author concludes in view of all his observations that in *Lathyrus odoratus* this phenomenon may occur in an early stage of prophase as well as at the time of disjunction according to different cases.

126. On the Configuration of Gemini in the Pollen Mother-cells of *Vicia faba*, L. Takeshige MAEDA. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B 5, 1930, 125–137, 11 text-figs.).

In the heterotype division of the pollen mother-cells of *Vicia faba* there are 6 gemini, of which the largest and most conspicuous one is M-geminus and the shorter ones are m-gemini. In the configuration of these gemini we observe a wide variation, inasmuch as the number of intersection points or the attachment of the component univalents in the prophase is very various. It varies from 1–6 in m-gemini and from 2–13 in M-geminus. The ratio between the means of such points of M-geminus and m-gemini is nearly equal to that between their respective length, which indicates that the variation of the intersection points above noted is merely a matter of chance.

127. A Contribution to the Phytogeography of the Island of Yakusima I. (Japanese). Genkei MASAMUNE. (Bot. Mag. Tôkyô 44, 1930, 43–52).

Yakusima which is a circular island of nearly 6½ km. circuit lies between 132°22′–130°40′ E.L. and 30°4′–30°27′ N.L. On the basis of the author's comparative studies of the distribution of the Conifers in that Island, the Luchu Archipelago as well as Formosa he comes to the conclusion that from the viewpoint of plant geography the sea (so-called "Sititônada") lying between the Islands Yakusima and Amami-Ôsima has no great importance as the boundary of plant distribution.

128. On New and Noteworthy Plants from the Island of Yakusima II. Genkei MASAMUNE. (Bot. Mag. Tôkyô 44, 1930, 219–221).

An enumeration of 6 plants from the Island Yakusima, of which 3 are new species, viz. *Cirsium yakusimense, Cymbidium Nagi-folium*, and *Plantago yakusimensis*.

129. Antigenic Properties of Tobacco Mosaic Juice. (With Japanese résumé). Takashi MATSUMOTO. (Jour. Soc. Trop. Agric. 1, 1930, 291–300).

If the juice of tobacco suffering from the mosaic disease is injected into the rabbits antibodies are formed in their serum which can exercise the specific action of precipitating the infective principle of the virus. Consequently when the mosaic juice to which the antiserum is added is filtered up, the supernatant liquids contain no infective principle of the virus, while the precipitate itself retains the infective principle quite unaffected, provided that the concentration of the serum is not extremely high or its reaction time not very long. Healthy serum has neither inhibiting nor impairing action on the virus.

130. Flora of Hokkaido and Saghalien. Kingo MIYABE and Yushun KUDO. (Jour. Fac. Agric., Hokkaido Imp. Univ. Sapporo **26**, 1930, 3 pp. preface and 79 pp. text).

The first part of the series containing an enumeration of plants in Hokkaido, Kuriles

and Saghalien with synonyms and literature. The present part contains the Pteridophytes and Gymnosperms, 126 spp. on the whole.

131. On the Inheritance of Variegation Disease in a Strain of Rice-plant. (Japanese). Isaburo NAGAI and Siroku HARA. (Japan. Jour. Gen. 5, 140–144, with figs.).

In a Corean race of rice-plant the authors have observed a disease which produces deep reddish brown panther-like spots and which is due to *Helminthosporium Oryzae*. According to the results of their observation in the first year healthy and diseased stocks were found in the ratio 3 : 1. In the second year seeds got on healthy stocks were sown and it was seen that certain families (16 in number) consist exclusively of healthy plants and certain others (22 in number) of the mixture of healthy and diseased ones. Seeds from diseased plants have given rise exclusively to diseased offspring. The hybridization healthy × diseased was observed to produce healthy offspring in F_1, and healthy and diseased in the ratio 3 : 1 in F_2. From all just stated it is clear that disease character is recessive to healthy and that the disease has appeared at first by mutation and given rise at the first year of the authors' observation to the heterozygous plant.

132. The Number of Chromosomes in the Cultivated Varieties of *Brassica*. (Japanese with English résumé). Keizo NAGAI and Tsunetaro SASAOKA. (Japan. Jour. Gen. 5, 1930, 151–158, 1 pl.).

The chromosome number in 13 species of *Brassica* incl. about 100 varieties was counted. The results are given in a table and 1 plate. The haploid number is 8, 9, 10, 12 and 18 in various species respectively.

133. On the Melosis in the Polyanthus Narcissus, *Narcissus Tazetta* L. Karyological Studies of the Narcissus Plant II. (Japanese with English résumé). Seijin NAGAO. (Japan. Jour. Gen. 5, 1930, 159–171, 22 figs.).

The chromosome number in root-tip cells of various garden varieties of the polyanthus narcissus is various. In Franklin and one other variety 10 gemini are observed in the pollen mother-cells, which shows that here 10 is the basic number. It may be added that the number 7 has been formerly given by the author in other varieties. In still another variety the somatic number was found to be 22. In the latter case 11 gemini are sometimes seen in the pollen mother-cells, but generally only 10 gemini are observed; this is due to the fact that while in the latter case two bivalents form one tetrapartite complex, in the former the cross segmentation occurs in this complex and gives rise to two separate chromosomes. In the variety Yellow Prince 10 trivalents are seen, and in Luna the meiotic division is so irregular that the number of chromosomes remains yet undetermined.

134. Chromosome Arrangement in the Heterotype Division of Pollen Mother-cells in *Narcissus Tazetta* L. and *Lilium japonicum* THUNB. Seijin NAGAO. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B 5, 1930, 163–182, 5 text-figs.).

In the heterotype division of the pollen mother-cells in *Narcissus Tazetta* the form of chromosome arrangement with two inner chromosomes, i.e. that resembling the stable form of floating magnets, is most frequent. Then comes in frequency the arrangement with three inner chromosomes. The small chromosomes tend to occupy the position inside

the chromosome ring. In the same process of *Lilium japonicum* with 12 gemini the case is most frequent, where 2 out of 12 chromosomes occupy the inner position instead of 3; this is the number for the arrangement resembling the stable form of floating magnets. As in *Narcissus*, so also in *Lilium* the small chromosomes tend to occupy the inner position.

135. On the Chromosome Number in Some Agricultural Plants. Goichi NAKAJIMA. (Japan. Jour. Gen. 5, 172–176, 20 text-figs.).

The chromosome number, somatic as well as reduced, in certain Gramineae and 2 *Plantago* species was determined.

136. Studies on *Sclerotium Rolfsii* SACC. Pt. 7. The Results of Successive Cultures and Selections within Pure Line of the Fungi. (Japanese with English résumé). Kakugoro NAKATA. (Bult. Sc. Fak. Terk. Kjusu Imp. Univ. 3, 1929, 291–299).

Plus, minus and mean selections for the diameter of sclerotia in a pure strain of *Sclerotium Rolfsii* were made during several generations. The fungus was cultivated in CZAPEK's agar. In these experiments minus selection especially seemed apparently to have been successful, because the size of sclerotia decreased gradually in each generation. According to the author's view this is however not at all the effect of selection, but simply due to the character of nutrient media. The koji agar contains a certain substance which stimulates the growth of mycelia and participates in the formation of sclerotia and which seems to correspond to the vitamine found by WILLAMAN in *Sclerotium cinerea*; the CZAPEK's agar does not contain this special substance and hence the culture of the fungus in this nutrient medium leads to the gradual diminution of the size of sclerotia in successive generations.

137. Comparative Studies of *Bacterium sesami, B. solanacearum* and *B. sesamicola.* (Japanese with English résumé). Kakugoro NAKATA. (Ann. Phytopathol. Soc. Japan 2, 1923, 229–243, 1 pl.).

The author has performed a series of culture experiments on *Bacterium sesami, B. solanacearum* and *B. sesamicola*, all of which are known to be the cause of the sesame disease. In view of the results of his investigations the author has expressed the opinion that *B. sesami* is identical with *B. sesamicola* and different from *B. solanacearum*. The characters of *B. sesami* MALKOFF (Syn. *Ps. sesami* MALKOFF, *B. sesamicola* TAKIMOTO), morphological, cultural as well as physiological, as revised by the author, are described in detail.

138. Ueber die Bildung von konzentrischen Zonen einiger *Helminthosporium*-Arten in Reinkulturen. Yosikazu NISIKADO. (Ber. Ôhara Inst. Landw. Forsch. 4, 1930, 349–355, 1 Taf.).

Die Reinkultur von *Helminthosporium*arten lässt nicht selten die Zonenbildung erkennen. Um die Ursache dieser eigentümlichen Erscheinung zu studieren, hat der Verf. betreffend die auf Reisstrohdekotagar kultivierten *Helminthosporium*arten (*H. Oryzae, Setariae, Maydis*, und noch eine andere) die folgenden Experimente ausgeführt. Bei den Dunkelkulturen bei 20° bzw. 30°C waren die Resultate negativ, während dieselben bei abwechselnd 22° und 30° die klare Zonenbildung erkennen liessen. Auch die Kulturen, welche abwechselnd belichtet und verdunkelt wurden, haben positive Resultate gegeben. Hinzuzufügen ist, dass die Natur der konzentrischen Zonen bei einzelnen Arten nicht einheitlich ist.

139. On the Systematic Importance of the Spodograms of the Leaves of the Bambusaceae (VIII). (Japanese). Kiichi OHKI. (Bot. Mag. Tôkyô 44, 1930, 351–359, with figs.).

The present part which is a continuation of the author's studies on the subject enunciated in the above title includes the genus *Phyllostachys*, incl. two species *reticulata* and *Makino*.

140. On a Fungus Found in the Urine and the Cerebro-spinal Fluid of a Patient Suffering from Meningitis. Toshio OHUE. (The Sc. Rpts. Tôhoku Imp. Univ. 4. Ser. (Biol.) 5, 1930, 117–132, 2 pls. and 3 figs.).

A species of the fungus was found in the urine of a patient suffering from meningitis. It is in all probability identical to *Alternaria tenuis* NEES, as recognized by its cultural and pathogenic characters. When a fluid retaining its suspension is injected into animals their mortality rate varies from 23% (albino rats) to 67% (guinea pigs). By the infection of sterilized culture of the fungus and living culture of *Alternaria* sp. on leaves of the poppy no remarkable disturbance was observed.

141. Ueber Parthenogenesis bei *Houttuynia cordata*. Sakuichi OKABE. (Japanisch m. deutsch. Zfg.). (Japan. Jour. Gen. 6, 1930, 14–19, m. 8 Textabb.).

Im Jahre 1908 haben SHIBATA und MIYAKE die parthenogenetische Entwicklung bei *Houttuynia cordata* erwiesen, und dabei wurde es zugleich festgestellt, dass die Chromosomenzahl in allen Generationen 52–56 beträgt und bei der Mitose der Pollen- und Embryosackmutterzellen die Chromosomen einfach sind und keine Reduktion stattfindet. Im Jahre 1927 hat SÖDERBERG die Tatsache veröffentlicht, dass bei der gleichen Pflanze die Chromosomenzahl 100–104 beträgt und bei der Kernteilung der Pollenmutterzellen man ungefähr 50 Gemini nachweisen kann, wodurch er das Geschehen der Parthenogenesis in dieser Pflanze für zweifelhaft hielt.

Nach den Verf.s neuen Studien beträgt die Chromosomenzahl 94–98. Bei der Kernteilung der Pollenmutterzellen kann man ausser vielen Gemini einige Univalente beobachten und die Chromosomenreduktion wird unregelmässig ausgeführt. Bei der Kernteilung der Embryomutterzellen treten in gewissen Fällen viele Gemini und einige Univalente auf und in den anderen sind alle ausnahmslos die Univalente; keine Chromosomenreduktion findet statt. Die parthenogenetische Entwicklung der Eizelle wurde auch experimentell nachgewiesen.

142. Study of *Euryale ferox* SALISB. V. On Some Features in the Physiology of the Seed with Special Respect to the Problem of the Delayed Germination. Yônosuke OKADA. (The Sc. Rpts. Tôhoku Imp. Univ. 4. Ser. (Biol.) 5, 1930, 41–116, 1 pl. and 4 text-figs.).

The seeds of *Euryale ferox* freshly harvested do germinate neither under ordinary room temperature of the laboratory nor under high temperature. When they are stored up in mud and water in a hothouse, some ones spring in the first spring after harvest, but the majority first germinates in the second spring, and the fact is noticed that smaller seeds tend to germinate earlier than the larger ones. For the germination of *Euryale* seeds light seems not to be specially necessary. Their germination is quite indifferent towards the oxygen relation. Seeds are rich in water and decortication effects no further

imbibition of water. The water can pass freely through the seed-coat, and it was concluded that the coat characters are not responsible for the delayed germination.

The embryo is fully established in seeds in differentiation and magnitude, and when isolated from dormant seeds it may be forced to germinate by cultivating it in a sugar-containing medium, and even under anaerobic condition. Various experiments were made to force the germination of seeds, of which the cold storage is most promising.

143. On the Evolution of Classification of the Rhodophyceae. (Japanese). Kintarô OKAMURA. (Bot. Mag. Tôkyô 44, 1930, 61–69).

The word Florideae was first created in 1813 by LAMOUREUX, though his so-called Florideae contained besides the red algae in modern sense many plants which are not. The foundation for the modern classification of red algae was laid by BORNET and THURET, who have first recognized the female nature of the carpogonium. J. G. AGARDH's classification was long prevalent. SCHMITZ published in 1883 his system of the Florideae (except the Bangiales), which included Nemalioninae, Gigartininae, Rhodymeninae and Cryptomeninae. OLTMANNS (1904) distinguished five groups, viz. Nemalionales, Cryptomeniales, Ceramiales, Gigartinales and Rhodymeniales, though later the order of these groups was changed. KYLIN (1928) has separated certain forms from Nemalionales and created a new group Gelidiales.

144. Ueber die intersexuellen Pflanzen von *Rumex Acetosa* L. (Japanisch). Tomowo ONO. (Japan. Jour. Gen. 5, 1930, 118–120).

Wenngleich *Rumex Acetosa* gewöhnlich diözisch ist, kann man davon bisweilen intersexuelle Pflanzen finden. Dabei beobachtet man im allgemeinen an ein und demselben Stocke männliche und hermaphrodite, oft wenige weibliche Blüten. Bei den hermaphroditen Blüten gibt es ausser den normalen Staubblättern und Fruchtknoten die in verschiedenen Reduktionsstadien befindlichen. Wenn man die männlichen, weiblichen und intersexuellen Pflanzen zueinander vergleicht, so sieht man, dass die Höhe der Blütenschaften, der Grad der Verzweigung, sowie die Grösse der Spaltöffnungen an den Blättern am grössten bei den weiblichen, am kleinsten bei den männlichen und mittelmässig bei den intersexuellen Individuen sind.

145. Further Investigations on the Cytology of *Rumex* VI. On the Intersexual Plant of *R. Acetosa*.—VII. Chromosomes of *R. montana*.—VIII. Chromosomes of an Intersexual Plant of *R. acetosella*. (Japanese with English résumé). Tomowo ONO. (Bot. Mag. Tôkyô 44, 1930, 18 text-figs.).

An intersexual plant of *Rumex Acetosa* bears generally male and bisexual, and also female flowers. The bisexual flowers are found most frequently on triploid plants ($3n=22$); their number varies from almost 0 to 98%.

The chromosome number of *R. montanus* is

$$♀ = 14 = 12 + 2X, ♂ = 15 = 12 + X + 2Y$$
$$☿ = 22 = 18 + 2X + 2Y$$

In the intersexual plant of *R. acetosella* the author has counted 20 bivalents and 1 univalent in the heterotype nuclear division of pollen mother-cells.

146. Chromosomenmorphologie von *Rumex Acetosa*. Tomowo ONO. (The Sc. Rpts. Tôhoku Imp. Univ. 4. Ser. (Biol.) 5, 1930, 415–422, 21 Textabb.).

Die vorliegende Mitteilung bezieht sich auf die Gestaltverhältnisse der Chromosomen bei *Rumex Acetosa*. Bei männlichen Pflanzen (15 = 12 + X + 2Y Chromosomen) ist das X das längste Chromosom mit einer Einschnürung in der Mitte, während jedes Y kürzer als X und zweischenkelig ist. Die Autosomen stellen gerade Stäbchen dar. Die Chromosomen bei weiblichen Pflanzen ähneln ungefähr denselben der männlichen. Bei den 15- und 22-chromosomigen intersexuellen Pflanzen hat der Verf. ein verhältnismässig kurzes zweischenliges Autosom gefunden, welches sogar auch bei einigen diploiden weiblichen Pflanzen beobachtet wurde. Weder bei 21-chromosomigen intersexuellen noch bei tetraploiden Pflanzen wurde diese eigentümliche Autosom beobachtet.

147. Notes on Some Fossil Plants from the Upper Triassic Beds of Nariwa, Prov. Bitchû, Japan. Saburô ÔISHI. (Japan. Jour. Geol. & Geor. 7, 1930, 49, 1 pl.).

The following fossil plants are described: *Annulariopsis inopinnata* ZEILLER(?), *Hausmannia nariwaënse* n. sp., *Dictyophyllum* sp., *Dictyophyllum*(?) sp., *Baiera* sp., *Baiera* sp. cfr. *B. paucipartita* NATHORST, *Podozamites* sp.

148. Critical Considerations on the Phylogenetic System of Classification of Plants. I. On the Diagnostic Character of Phylum and Subphylum. (P. N.). (Japanisch). Mitiharu SAKISAKA and Yositô SINOTÔ. (Bot. Mag. Tôkyô 44, 1930, 285–298, 2 Textabb. m. vielen einzelnen Fig.).

Nicht befriedigt mit den bisher zu Tage getretenen Klassifikationsystemen der Pflanzen verschiedener Forscher schlagen beide Verf. ein neues vor, welches in erster Linie auf die Zahl und Insertionsweise der Zilien bei den Gameten und Agameten begründet ist. Danach wird das ganze Pflanzenreich (ausgenommen die Schizophyten) zu 4 Stämmen (Phylum) (Phylum di-, gastro-, mono- und polycontophyta) eingeteilt. Jedes Stamm wird weiter zu den Abteilungen verschiedener Ordnungen zergliedert. Ein Beispiel folgt unten:

III. Phylum. Monocontophyta
- A. Monoflagellata
- B. Monoconta
  1. Subphylum. Monocontae: Myxomycetes, Archimycetes, Oomycetes.
  2. Subphylum. Amonocontae: Bangiales, Florideae, Ascomycetes, Basidiomycetes.

149. Karyological Observations in Different Interspecific Hybrids of *Brassica*. Tsunetaro SASAOKA. (Japan. Jour. Gen. 6, 1930, 20–32, 72 figs.).

The hybridization between various species of *Brassica* were performed. In those between the species showing 19 and 10 chromosomes in their respective pollen mother-cells (either 19 × 10 or 10 × 19) 10 bivalents and 9 univalents appear in the heterotype division; the bivalents are separated regularly and the univalents, of which a few are split, are distributed irregularly. In the hybrids between the species having 19 and 18 chromosomes (19 × 18 or 18 × 19) 10 bivalents and 17 univalents appear in the heterotype division. In the two above cases the homotype division was quite normal, though in the heterotype division in F_2 generation abnormalities were again observed. In the hybrids between the species, both having 19 chromosomes, no irregularities in the division were observed.

150. Systematic Importance of Spodograms of Leaves in the Urticales. III. Yosisuke SATAKE. (Bot. Mag. Tôkyô 44, 1930, 113–122, 3 text-figs.).

Continuation of the author's studies on the subject above enunciated. This part contains various species belonging to the genera of the Urticaceae, incl. *Parietaria, Memorialis, Pouzolzia, Debregeasia, Villebrunea, Boehmeria, Pipturus, Laportea, Sceptrocnide, Nanocnide, Urtica, Pilea, Achudenia, Pellionia, Elatostema.* The distinction is based on the characters of cystoliths, epidermal cells and hairs, and also on the presence of calcium oxalate crystals. The paper ends with a short discussion on the importance of spodograms in the classification of plants in general.

151. Untersuchungen über die Bedeutung des Cytochroms in der Physiologie der Zellatmung. Keita SHIBATA und Hiroshi TAMIYA. (Acta Phytochimica, 5, 1930, 23–97, m. 15 Abb.).

Die Verfasser haben die physiologische Bedeutung des von KEILIN entdeckten Cytochroms experimentell aufzuklären versucht. Als Versuchsmaterial diente hauptsächlich die Bäckerhefe. Im Gegensatz zu der Annahme KEILINs haben die Verfasser zunächst nachgewiesen, dass die Bindung des Cytochroms mit Sauerstoff ganz unabhängig von der Oxydasewirkung stattfindet. Andererseits wird die Entbindung des vom Cytochrom gebundenen Sauerstoffs durch blosse O_2-Entziehung zuwege gebracht, woraus der Schluss gezogen wird, dass nämlich die Bindung des Cytochroms mit Sauerstoff keine echte Oxydation, sondern ein Oxygenierungsvorgang wie beim Hämoglobin darstellt. Aus diesem Befund wird die Annahme nahegelegt, dass die oxygenierte Form des Cytochroms (Oxycytochrom), wie das normale Cytochrom, eine Ferro-Verbindung ist, und ferner, dass der vom Cytochrom gebundene Sauerstoff kein aktiver, sondern ein molekularer ist. Es wurde nachgewiesen, dass die Oxygenierung aller drei Komponenten des Cytochroms durch Zusatz einer kleinen Menge von KCN stark gehemmt wird, und ferner, dass die Oxygenierung des Cytochroms bei niedriger Temperatur mit viel grösserer Reaktionsgeschwindigkeit vor sich geht als bei höherer Temperatur.

Die Oxygenierungsfähigkeit des Cytochroms wird bei Kochen, Trocknen, Acetonbehandlung, Zusatz von Oxydationsmitteln, Alkali oder Butylalkohol und Urethan in höherer Konzentration zerstört, wobei je nach den Bedingungen denaturierte Hämochromogen (Fe″)- oder Hämatin (Fe‴)-Derivate der drei Cytochromkomponenten gebildet werden. Die echte Oxydation der aus Cytochrom abgeleiteten Hämochromogen-Derivate zu entsprechenden Ferri-Verbindungen wird durch KCN gar nicht gehemmt. Die Ferri-Derivate des Cytochroms kann man nicht in ursprüngliches normales Cytochrom verwandeln.

Theoretisch haben die Verfasser gefolgert, dass das Cytochrom für die Zellen, bei denen die Menge des durch freies Diffundieren dem Zellplasma zugänglichen Sauerstoffs der Atmungsintensität des Plasmas gegenüber genügend gross ist, nicht notwendig vorhanden sein muss, und dass andernfalls das Vorhandensein des Cytochroms unumgänglich notwendig wird. Damit steht im Einklang die Tatsache, dass die an freier Luft vegetierenden Aerobien, wie Schimmelpilze, nur wenig oder oft gar kein Cytochrom enthalten, obwohl ihre Atmungsintensität derjenigen der Hefe- oder Bakterien, die submers unter erschwerten Sauerstoffzufuhr leben und demzufolge mit Hilfe des Cytochroms atmen, kaum nachsteht. Dank der starken Oxygenierungsfähigkeit des Cytochroms gehen die Atmung der Bäckerhefen und aeroben Bakterien in weiten Grenzen unabhängig von der O_2-Spannung im Aussenmedium vor sich, während die Atmung der cytochromfreien oder -armen Organismen wie Schimmelpilze dem schwankenden O_2-Druck gegenüber sehr

empfindlich ist.

Die Atmung der cytochromfreien Zellen wird durch Kohlenoxyd gar nicht gehemmt, während diejenige der cytochromreichen Zellen dadurch mehr oder minder gehemmt wird. Unter Hinweis auf verschiedene Umstände wurde von den Verfassern die Ansicht vertreten, dass das „Atmungsferment" von WARBURG wahrscheinlich nur ein Synonym des Cytochroms sei.

Aus zwei Arten von *Lactarius* haben die Verfasser eine typische indophenolbildende Oxydase extrahiert. Obwohl die Wirkung dieser Oxydase in vitro durch H_2S, KCN und Acetonbehandlung vollständig aufgehoben wird, übt Kohlenoxyd darauf gar keinen Einfluss. Die intrazellulare Oxydase der Hefezellen verdankt aber den für ihre Wirkung notwendigen Sauerstoff ganz und gar dem oxygenierten Cytochrom, und die Oxydasereaktion bei intakten Hefezellen durch CO deutlich gehemmt wird. Diese Tatsache bietet also einen triftigen Grund dafür, dass das Kohlenoxyd auf die Oxygenierungsfähigkeit des Cytochroms hemmend wirkt.

Bei Vertretern mehrerer Tierstämmen (Poriferen, Coelenteraten, Echinodermen und Tunicaten) wurden das Cytochrom bzw. die Hämochromogene hier zum ersten Mal nachgewiesen. Unter phylogenetischen sowie ökologischen Betrachtungen haben die Verfasser die Ausbildung und Bedeutung verschiedener Atmungspigmente im Pflanzen- und Tierreich näher besprochen, wobei das an Zellplasma festsitzende Cytochrom als der Prototyp aller O_2-speichernden oder O_2-Druck regulierenden Pigmente in Vordergrund des Interesses gestellt wurde. Verfasser.

152. On the Spiral Structure of Chromosomes in Some Higher Plants. Namio SHINKE. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B 5, 1930, 239–245).

Concerning a certain number of plants belonging to the genera *Cryptomeria, Sagittaria, Secale, Lathyrus,* etc., etc. the spiral structure of chromosomes was demonstrated, and in many cases it was traced through various stages of the meiosis in the pollen mother-cells.

153. The Germination Test of Pollen in Some Vegetable Crops with Special Reference to the Influence of the Hydrogen-ion Concentration of the Media. Makoto SISA. (Jour. Sc. Agric. Soc. No. 323, 1930, 88–94).

The percentage of pollen germination of tomato, cucumber, and *Brassica japonica* var. *Suigukina* was tested on agar media under varying conditions, especially under different H-ion concentrations. In tomato glucose is more favourable for germination than sucrose. In respect to H-ion concentration two optima were observed, viz. pH 5.5 and 6.4, the minimum lying at 5.9. The influence of Cl- or Na-ion was negligible in the author's experiments. In pollen of cucumber, the highest percentage of germination in sucrose agar was found at pH 5.5, while in that of *Brassica* the optimum for germination in sucrose agar lies at pH 7.7.

154. Ueber die infolge des Wechsels der Aussenbedingungen ausgebildeten embryosackartigen Pollenkörner. (Japanisch). Isamu STOW. (Japan Jour. Gen. 5, 1930, 145–146).

Beim Unterwerfen von Victor, einer Rasse von *Hyacinthus orientalis* unter eine hohe Temperatur zur Zeit der Reduktionsteilung der Pollenmutterzellen ist es dem Verf. gelungen, die mehrkernigen Pollenkörner von kolossaler Grösse zu bekommen. Ihre

Herkunft ist wie folgt. Nach der gewöhnlichen Tetradenbildung vergrössern sich die Pollenkörner etwas, von denen bei jedem drei sukzessive Kernteilungen erfolgen, um 8-kernige Körner zu produzieren. Unter diesen acht Kernen sitzen je drei an beiden Enden jedes Kornes und zwei übrigen zwischen beiden. Um den ersteren Kernen sind die aus Pektin bestehenden Zellmembranen produziert, womit die ganze Struktur völlig mit derselben des Embryosacks übereinstimmt. Die zwei übrigen Kerne schmelzen oft zusammen, ähnlich den Polkernen des Embryosackes. Hinzuzufügen ist, dass für die Produktion solcher abnormen Pollenkörner das Aussterben vieler anderen Pollenkörner eine notwendige Bedingung ist.

155. Experimental Studies of the Possibility of Primary Infection by *Piricularia Oryzae* and *Ophiobolus Miyabeanus* hibernating in Rice Seeds. (Japanese with English résumé). Hashio SUZUKI. (Ann Phytopath. Soc. Japan **2**, 245–275, 1 pl.).

When the rice seeds are infected by *Piricularia Oryzae* or *Ophiobolus Miyabeanus* they show generally a visible discolouration, but sometimes no external signs of infection are visible. Both fungi have the power of infecting seeds at the time before and after the flowering period. The fungi which have hibernated in seeds are able to infect the young seedlings soon after germination. *Piricularia* and *Ophiobolus* hibernating in rice seeds may live at least for two and four years respectively, but will soon die after 5 min. in hot water kept at 50° and 55°C respectively.

156. On the Sex-chromosome of *Rumex montanus* DESF. (Japanese with English résumé). Yô TAKENAKA. (Bot. Mag. Tôkyô **44**, 1930, 176–184, 3 figs.).

The cytological studies on *Rumex montanus* concerning the chromosome number made independently led the author to almost the same conclusion as ONO (cf. No. 145), viz.

		diploid	haploid
♂	plant	$15=12+Y_1+X+Y_2$	$6+X,\ 6+Y_1+Y_2$
♀	„	$14=12+X+X$	$6+X,\ 6+X$

157. Anatomische Studien über die Pflanzen, von denen das Pfropfen schwer oder leicht auszuführen ist. (Japanisch). Yoshio TAKENOUCHI. (Agric. & Hortic. **5**, 1930, 145–152 m. Abb.).

Das Pfropfen gelingt leicht beim Maulbeerbaum, dagegen sehr schwierig beim Mangobaum, *Nephelium Longana*, Teebaum, *Psidium guyava*, während der Birnbaum und verschiedene *Citrus*arten in dieser Hinsicht mittelmässig stehen. Aus der Vergleichung der obengenannten Pflanzen ergibt es sich, 1. dass die Holzgefässe weitlumiger und weniger pro 1 mm² vertreten sind beim Teebaum als beim Maulbeerbaum, Birnbaum, *Nephelium*, Mangobaum, während die *Citrus*arten eine Zwischenstufe von beiden darstellen, 2. dass beim Birnbaum usw. die sekundären Rindenzellen länger als breit sind, während es beim Teebaum usw. ganz umgekehrt ist, 3. dass je leichter das Pfropfen auszuführen ist, desto kleiner die Gerbstoffmenge ist, 4. dass die osmotische Druck der Rinderzellen grösser ist bei den schwer aufzupfropfenden Pflanzen als bei den anderen, und 5, dass die Saugkraft der Wurzel beim Maulbeer- und Teebaum 6–8 bzw. 11 Atm. beträgt, während sie bei *Citrus* ungefähr 5 Atm. nicht überschreitet.

158. Studien über die Schnittfläche der Pfropflinge. (Japanisch). Yoshio TAKENOUCHI. (Agric. & Hortic. **5**, 1930, 429–438).

Unter den von dem Verf. zur Untersuchung genommenen Pfropflingen war der an der Schnittfläche ausgebildete Callus beim Birnbaum der grösste und beim *Nephelium Longana* der kleinste, während er beim Maulbeer-, Mango-, Teebaum usw. in seiner Grösse zwischen beiden Extremen stand. Das Zellteilungsvermögen wird beim Mark am kürzesten, beim Holz viel länger und bei dem Cambium und der Rinde am längsten beibehalten. Das Wundkernholz wird an der Schnittfläche von *Tea* und *Nephelium* am reichlichsten, an derselben von *Morus, Pirus, Psidium* usw. weniger reichlich ausgebildet. Die Menge der Oxydase an der Schnittfläche ist bei *Morus, Citrus, Tea* ziemlich gross und bei *Pirus, Psidium, Nephelium,* Mango relativ klein. Die Sauerstoffmenge ist bei *Morus,* Mango *Tea, Psidium* verhältnismässig reichlich, bei *Citrus* und *Nephelium* wenig. Wenn man die Schnittfläche durch gewisse Agentien behandelt, um die Callusbildung zu beschleunigen, ist der Effekt nach den Agentienarten verschieden.

159. *Penicillium* Rots of *Citrus* Fruits. (Japanese with English résumé). Haruyoshi TAKEUCHI. (Bul. Sc. Fak. Terk. Kjusu Imp. Univ. **3**, 1929, 333–449).

The following species of *Penicillium* were recognized as the cause of the rot of *Citrus unshiu*, viz. *P. italicum, digitatum, expansum* and *fructigenum* (n. sp.). Of the above species the first two are parasitic exclusively on *Citrus* fruits, but others, besides on the latter, also on apples, pears, persimmons and grapes. The description of *P. fructigenum* is given.

160. Ueber die Minimum-Temperatur des Aufblühens und der Fruchtbildung beim Sumpfreis. (Japanisch). Yosuke TAKIGUTI. (Agric. & Hortic. **5**, 1930, 165–171).

Um die Minimum-Temperatur des Aufblühens beim Sumpfreis zu bestimmen hat der Verf. die ganze Pflanze in einem Zimmer mit konstanter Temperatur gesetzt. Dabei wurde es gefunden, dass sie für das Oeffnen der Blüten 15°C und für das Gelingen der Bestäubung 18°–19°C beträgt.

161. On the Pathogenicity of *Typhula graminum*, KARSTEN. (Japanese with English résumé). Heizi TASUGI. (Jour. Imp. Agric. Exp. Sta. **1**, 1903, 183–198, 2 pls.).

The author has performed the inoculation experiments of barleys, both naked and hulled, as well as wheats with *Typhula graminum*, the causal organism of snow-rot. When these cereals are inoculated in their young stage, their death and subsequent rot are the necessary consequences. Th attack is much terrible when plants are immersed in water under low temperature. Six strains of the fungus isolated from hulled and naked barley, wheat, *Eleusine indica* and *Alopecurus fulvus* respectively were observed to attack both barley and wheat, though the latter is most resistant against the attack. The snow was often regarded as the cause of the rot, but the author thinks that *Typhula graminum* is the true cause of the disease, low temperature and moist condition much favouring the infection.

162. Vergleichende Studien über die Säurebildung, die Atmung, die Oxydasereaktion und das Dehydrierungsvermögen von *Aspergillus*arten. Hiroshi TAMIYA und Tatsutaro HIDA. (Acta Phytochimica **4**, 1929, 343–361, 1 Abb.).

An einer Anzahl von *Aspergillus*arten haben die Verfasser die Säurebildung, die Atmung, die Oxydasereaktion und das Dehydrierungsvermögen vergleichend untersucht.

Auf einer N-armen und mit $CaCO_3$ beschickten Nährlösung wurde Glukonsäure nahezu von allen, besonders aber von *Asp. Awamori, flavus* und *gymnosardae*, gebildet, nicht aber von *Asp. soya* und *ostianus*. Citronensäure wurde auch bei vielen Arten (besonders deutlich bei *Asp. aureus, Awamori* und *niger*) nachgewiesen, während Oxalsäure nur bei einigen wenigen Arten gebildet wurde. Die bisher nur selten beobachtete Kojisäure wird von vielen *Aspergilli* gebildet und zwar in folgender Reihenfolge:

oryzae, flavus var.>*gymnosardae*>*Awamori, candidus, clavatus, flavus, fumigatus, giganteus.*

Die Atmungsintensität wurde mittels 2–4 Tage alten, auf Pfefferscher Nährlösung gezüchteten Decken durch Bestimmung des O_2-Verbrauchs ermittelt, wobei zugleich der Aufbauquotient (Pilzgewichtzunahme in g/veratmeter Zucker in mg) bestimmt wurde. In Bezug auf die Atmungsintensität und den Aufbauquotient bestehen innerhalb der verschiedenen *Aspergilli* gewisse Unterschiede. Während der Aufbauquotient im Laufe der kurzen Kulturdauer nahezu unverändert bleibt, steigen die Atmungsintensität während der Wachstumszeit von 3–6 Tagen fast immer ab.

In Bezug auf die Intensität der Indophenolasereaktion erhielten die Verfasser folgende Reihenfolge:

giganteus>*Onikii, flavus*, var., *gymnosardae*>*oryzae melleus* >*ochraceus, Awamori, soya*>*clavatus*>*aureus, niger.*

Die Oxydasereaktion von allen untersuchten *Aspergilli* wurde beim Erwärmen der Hyphensuspension auf 52–65° deutlich herabgesetzt. Auch bei Störung des Reduktionssystems der Zelle durch Urethanzusatz wurde nicht nur keine Steigerung, sondern oft eine mehr oder weniger starke Hemmung der Oxydasereaktion beobachtet. Die Wirksamkeit der Oxydase ist bei jüngeren Decken grösser als bei älteren, und nimmt bei Anaerobiose der Decke sehr schnell ab.

Das Mb-Reduktionsvermögen der Pilzdecke äussert sich auch je nach der Pilzart sehr verschieden, am stärksten bei *Asp. clavatus*, darauf folgt *Asp. aureus, oryzae, Awamori, soya* u.a., und am schwächsten bei *Asp. Onikii, niger* und *ochraceus*. Durch Zusatz des Succinates stieg das Mb-Reduktionsvermögen von mehreren *Aspergilli* fast auf das Doppelte, während das Vorhandensein der Citricodehydrogenase bei allen untersuchten *Aspergilli* nicht nachzuweisen war. Die Kulturlösung von *Asp. oryzae* und von *Asp. gymnosardae* zeigten eine Fähigkeit, das zugesetzte Methylenblau zu entfärben, während bei anderen *Aspergilli* (*Awamori, aureus, clavatus, flavus, giganteus* u.a.) auch bei Zusatz von Succinat gar keine Entfärbung des Methylenblaus erzielt wurde.

Zwischen den untersuchten oxydativen Stoffwechselvorgängen lässt sich hieraus vorläufig kein merklicher quantitativer Zusammenhang oder gar kein Parallelismus feststellen. Verfasser.

163. Bibliographie von *Aspergillus* 1729 bis 1928. Hiroshi Tamiya und Shinkichi Morita. (Bot. Mag. Tôkyô **44**, 1930, 1–7, 79–89, 139–150, 209–218, 251–261, 305–316).

Forsetzung beginnt mit Nr. 1656 und schliesst mit Nr. 2424. Das Autorenregister wird demnächst folgen.

164. Cytological Irregularities in Hybrids between Species of Wheat with the Same Chromosome Number. P. Thompsom and H. T. Robertson. Cytologia **1**, 1930, 252–262, 1 pl.).

In hydrids between different 42-chromosomic species of wheat (*vulgare* series) one

or a few univalent chromosomes are found lagging in the pollen mother cells concerned in division. Though such is occasionally the case also in pure species, the percentage of its occurrence is far larger in the former than in the latter case: for instance, in *vulgare* and *Spelta* it is 4.0 and 4.4% respectively, while in *vulgare* × *Spelta* it amounts to 42%. The fact may be interpreted either as the result of general weakened affinity associated with parental diversity or as the difference of some chromosomes between the two parents and the consequent difficulty of their mating.

165. Studies on the Disease of Fruit- and Useful Trees due to *Valsa*. (Japanese). Kogo TOGASHI. (Rpt. of Saitô Hôon Soc. 4, 1929, 229–234, 291–294).

The fungi belonging to *Valsa* which cause the canker or die-back disease of peach-trees are distinguishable into two types which are morphologically different. They differ by the size of stroma, asci, the temperature relation, etc. In respect to the *Valsa* species on some species of *Chamaecyparis* and *Thuyopsis* we may distinguish a certain number of different types.

As to two types of *Valsa* species on peach-trees above mentioned the author has enumerated some differences: for instance, one can infect not only peaches, but also plums, one variety of cherry, while the other *Prunus Mume*, but not plum, etc. , etc.

166. Morphological Studies of *Leucostoma leucostoma* and *Valsa japonica*, the Causal Fungi of Canker or Die-back Disease of Peach-trees. Kogo TOGASHI. (Bull. Imp. Coll. Agric. & Forest. Morioka 14, 1930, 50 pp. and 4 pls.).

Two fungi, viz. *Valsa leucostoma* (PERS.) FR. which is now better called *Leucostoma leucostoma* PERS. and *Valsa japonica* MIYABE et HEMMI are the cause of the canker or die-back disease of peach-trees prevalent in Japan. The author has studied about them the development of stromata, pycnidia, perithecia, etc., and besides he has performed the biometrical studies, of which the results are embodied in 31 tables.

In the stromata development of *Leucostoma leucostoma* it is remarkable that a cap of ectostromatic mycelium is always differentiated; it is composed of slender, colorless, rather prosenchymatous hyphal cells. This cap is considered as a distinguishing character of *Leucostoma* as a genus different from *Valsa*, where such a cap is never observed.

In *Leucostoma* the size of pycnospores is variable according to various seasons: their mean size as well as coefficient of variability being larger in August, September and October than in December. Furthermore, in both fungi, asci and ascospores are largest in late winter and early spring; when mature, they are very variable in their dimensions.

In a form of *Valsa japonica* on peach-trees the author has observed the fact that the ascus contains frequently less than eight ascospores and that a few of them are degenerated and sterile.

167. A New Species of *Urocystis* on *Convallaria majalis* L. Kogo TOGASHI and Fusaji ONUMA. (Japan. Jour. Bot. 5, 1930, 21–26, 1 text-fig.).

168. On the Medicinal Garden in Modern Ages and Koishikawa Medicinal Garden. (Japanese). Sanpei UEDA. (Bot. Mag. Tôkyô 44, 1930, 222–232).

A lecture given about the origin and history of medicinal gardens in Japan, especially on the Medicial Garden in Koisikawa, now the Botanical Garden of the Tokyo Imperial University. Its establishment dates back to 1684.

169. Mikrurgische Untersuchungen über die Entlassung der Spermatozoiden von *Isoetes*. B. WADA. (Cytologia **1**, 1930, 286–306, 2 Taf.).

Durch das Anstechen der Mikrosporen von *Isoetes japonica* mit Mikronadeln konnte der Verf. beobachten, dass die Spermatozoidenmutterzellen für das Wasser permeabel sind, aber die Antheridienzellen nicht. In den Spermatozoidenmutterzellen bei den reifen Antheridien hat der Verf. eine homogene Schleimsubstanz entdeckt, welche nach den mikrochemischen Studien Verfs. aus Pektinsubstanz bestehen soll, und welche in den zur Bauchseite der letzteren hin ausgedehnten Räumen enthalten ist. Bei der Entlassung der Spermatozoiden vergrössert sich das Volum dieser Substanz wegen der Wasseraufnahme und führt zum Oeffnen der Antheridien, wobei das Endosporium beim Bauchkamm platzt und die Spermatozoiden hinausgestossen werden. Bei den Anstichversuchen lassen sich die chloroformierten Spermatozoiden ebenso gut wie die normalen entlassen. Jedes Spermatozoid besitzt eine eigene Hülle, welche für ihre Aktivierung eine wichtige Rolle spielt; es beginnt an zu schwimmen nach deren Auflösen.

170. Ueber einige in Koisikawa botanischen Garten gesammelte *Isaria*-Arten. (Deutsch u. Japanisch). Eijiro YAKUSHIJI und Masao KUMAZAWA. (Bot. Mag. Tôkyô **44**, 1930, 40–42, 69–74, 1 Taf. und 2 Textabb.).

Drei Arten sind erwähnt, von denen eine, *I. nigra* neu ist und beschrieben wird.

171. Ein Beitrag zur Kenntnis der Gattung *Rhizopus*. I. Yoshihiko YAMAMOTO. (Jour. Fac. Agric., Hokkaido Imp. Univ. Sapporo, **28**, 1930, 1–101, 4 Tafeln und 16 Kurven).

Die Einleitung enthält Allgemeines über die Gattung *Rhizopus*. In Hinsicht auf 5 von dem Verf. selbst isolierten sowie 50 aus den anderen Forschern bezogenen und von ihm weiter gezüchteten Arten sind die morphologischen Merkmale ausführlich beschrieben, d.h. betreffend Rasen, Sporangienträger, Sporangien, Kolumellen, Sporen, Gemmen, Ausläufer, Rhizoiden, Hefezellen und Zygosporen. Dabei hat der Verf. gezeigt, dass der morphologische Charakter unter verschiedenen Aussenbedingungen stark schwanken kann, sodass es nur wenige konstante morphologische Charaktere gibt, welche für die Artunterscheidung benutzt werden können. Auf Grund solcher wenigen Merkmale hat der Verf. 2 Sektionen, Eurugorhizopus und Dubiorugorhizopus unterschieden, von denen jede je 3 untergeordnete Gruppe enthält.

Zum Schlusse hat der Verf. eine ausführliche Diagnose von 15 Arten angegeben.

172. Contributiones ad Floram Formosanum, II–III. Yoshimatsu YAMAMOTO. (Trans. Nat. Hist. Soc. Formosa **20**, 1930, 38–42, 97–105).

Unter 17 hervorgehobenen Pflanzen sind die folgenden neu: *Calanthe Kazuoi, Cymbidium linearisepalum, Plantago major* var. *Sawadai, Equisetum ramosissimum* var. *taikankoense, Aconitum Bartletti, Clematis Bartlettii, Euphrasia Matsudae, E. nankotaizanensis.*

173. Parthenocarpy Caused by the Stimuli of Pollination in Some Plants of Solanaceae. (Japanese with English résumé). (Prel. Rpt). Sadao YASUDA, Teturô KOMATU and Tosio NONOMURA. (Agric. & Hortic. **5**, 1930, 287–294 with 4 figs.).

The authors got parthenocarpic fruits of some Solanaceae by pollinating them with some other species. For instance, egg plant by *Petunia* sp., tomato by egg plant,

Capsicum sp. by *Physalis* sp., very rarely by *Petunia* or *Nicotiana* sp. The pollination of *Petunia* by egg plant, that of egg plant by *Petunia* sp. or by tomato, and that of *Capsicum* by egg plant or tomato always gave negative results.

174. On the Black-spot Disease of *Allium fistulosum*. (Japanese). Hazime YOSHII. (Jour. Plant Prot. **16**, 1929, 6 pp.).

The disease is due to *Macrosporium porri* ELLIS.

175. Leaf-spot Disease of *Astragalus sinicus*. (Japanese). Hazime YOSHII. (Jour. Plant Prot. **16**, 1929, 5 pp.).

Thyrospora astragali n. sp. is the causal organism of the disease. The diagnosis is given.

176. Black-spot Disease of Carrots. (Japanese). Hazime YOSHII. (Jour. Plant Prot. **16**, 1929, 4 pp.).

The causal fungus is *Alternaria radicina* M., D. et E. Long elliptical black spots are produced on the surface of carrot roots.

177. Summer Pest of Potatoes. (Japanese). Hazime YOSHII. (Jour. Plant Prot. **16**, 1929, 7 pp. with figs.).

According to the author's view the causal fungus of this disease should be *Macrosporium Solani* E. et M.

178. A Leaf-spot of Blight Disease of *Ricinus communis* L., Caused by *Macrosporium ricini* n. sp. (Japanese with English résumé). Hazime YOSHII. (Bul. Soc. Fak. Terk. Kjušu Imp. Univ. **3**, 1929, 327–332, 1 pl.).

The disease of *Ricinus communis* described in this paper is characterized by the production on leaves of an irregular cinnamon buff spot 10–20 cm in diameter, variegated with irregular towny olive zones, often surrounded by an etiolated halo. The disease is shown to be caused by *Macrosporium ricini* n. sp. by means of inoculations. The description of the causal fungus is given.

179. On a Bacterial Spot Disease of *Ricinus communis*, and its Causal Organism. (Japanese). Hazime YOSHII and Seito TAKIMOTO. (Jour. Plant Prot. **15**, 1928, 7 pp.).

On the basis of morphological and physiological characters elucidated by culture experiments the causal organism of the disease is called *Bacterium ricini*, of which the group number is 211.3232513.

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Hybridization between Old World and New World Cotton Species and the Chromosome Behavior of the Pollen Mother-Cells in the F_1-Hybrid

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With Plates VII-VIII and 7 Text-Figures

(Received April 10, 1931)

Introduction

Although no satisfactory botanical classification of the genus *Gossypium* has yet been made, cultivated cotton may be divided into two distinct groups, namely, those of the Old World and those of the New World. Numerous Asiatic forms which belong to the Old World group have been described as species, such as *G. herbaceum*, *G. Nankking*, *G. arboreum*, etc., each of which has 13 chromosomes in haploid number. American and Egyptian cottons which have 26 as the reduced number of chromosomes are described as species, such as *G. hirsutum*, *G. barbadens*, *G. brazilienses* etc., and belong to the New World cotton.

Many crossing experiments between Asiatic and American cottons have been tried with unsuccessful results since the 19th century by Trevor CLARKE and others. It has usually been held that it is practically impossible to secure a cross between the Asiatic (*G. herbaceum*) and American (*G. hirsutum*) cotton or between Egyptian and Asiatic cotton plants. Hybrids between Egyptian and American cotton, however, can be easily obtained. Recently, G. S. ZAITZEV reported that he had succeeded in his crossing experiments between Asiatic and American cotton by removing the staminal column sheath of the flower and then pollinating the stigma.

In all of his crossing experiments, it was stated that the pollination of Asiatic cotton by American cotton led more often to the formation of well developed ovaries than when the pollen of the Asiatic cotton was applied to the stigma of the American cotton.

He described the characteristics of the F_1 hybrid between Asiatic and American forms when Asiatic cotton was used as the female parent. In crossing experiment between *G. herbaceum* and *G. hirsutum*, B. B. DESAI painted the stigmas with various solutions before pollination and stated that the most successful results were obtained by treating the stigma prior to pollination with a dilute solution of cane sugar and citric acid. No report, however, has yet been made regarding the F_1 hybrid when the American cotton was used as the female parent.

For the purpose of studying the cytological as well as morphological characters of interspecific hybrids of cotton, several crossing experiments between Asiatic and American and between Egyptian and Asiatic cotton have been made by the author since 1927. Two F_1 hybrids between American and Asiatic cotton were obtained in 1928, and one F_1 plant between Egyptian and Asiatic cotton plant in the summer of 1930. All of these F_1 hybrids were successful when American and Egyptian cotton plants were used as the female parent.

The results of these crossing experiments indicate that it is not impossible to secure the F_1 hybrid even when American or Egyptian cotton plants are used as the female parent.

The most distinguishing in feature of these three F_1 plants was their perfect sterility, not only self-fertilization but also in fertilization with the pollen of the parents and of many other varieties. The development of the hybrids was much more vigorous than that of their parents, showing heterosis, and apparently their flowering did not show any abnormalities, though the development of the pollen grains was quite abnormal according to our observation.

VARIETIES USED IN HYBRIDIZATION EXPERIMENTS

1. Asiatic cotton (*G. herbaceum*):
   Local name Manshu-Zairaimen (black seeds) which is cultivated in South Manchuria and clearly distinguishable from the American cotton plant.

2. American cotton (*G. hirsutum*):
   Local name Reihomen, the seeds of which were imported from America and is now cultivated in the southern sections of China. The morphological characters are quite different from those of the Asiatic cotton plant.
3. Egyptian cotton (*G. barbadens*):
   Variety Aschmouni, the seeds of which were imported from Egypt and is grown in our Experiment Station.

The chief differentiating characters of these varieties are as follows:

	Color of petal	Length of fiber	Spot of petals	Flower-stalk	Chromosome number (haploid)
1. Asiatic	Yellow	24mm.	Deep	Curved downward	13
2. American	Cream	31	None	Erect	26
3. Egyptian	Yellow	35	Deep	Erect	26

Methods of Hybridization Employed

Special methods of crossing and chemical solutions were not used in any of our experiments. The day before the flower was to open, petals and bracts were first removed. The flowers were then emasculated and then covered with paraffin paper bags for protection against natural crossing. The pollination of the flowers was made from 10 a.m. to 2 p.m. the following day.

The following table gives the results of our crossing experiments.

TABLE 1

	Number of treated flowers	Number of mature bolls	Number of seeds	Number of germinated seeds
G. herbaceum × *G. hirsutum*	52	11	30	0
G. hirsutum × *G. herbaceum*	91	2	3	2
G. herbaceum × *G. barbadens*	21	0	0	0
G. barbadens × *G. herbaceum*	28	1	2	1

In the reciprocal crosses most of the treated flowers fell about a week after pollination, and only a few mature bolls were obtained. The seed, however, was very poor and only two germinated where in crossing *G. hirsutum* was used as the female paren and one seed where *G. barbadens* as the female parent.

In a crossing experiment between *G. herbaceum* and *G. hirsutum* G. S. ZAITZEV (1927) reported that it was more difficult to obtain F_1 seed when the pollen of the Asiatic cotton was applied to the stigma of American cotton than when Asiatic cotton was pollinated with American cotton.

In our crossing experiments between Old World and New World cottons, however, we succeeded in obtaining crosses when the New World cotton was used as the female parent. When Old World cotton was used as the female parent the percentage of the falling bolls in treated flowers was much smaller than that of reciprocal crosses, but the seed was sterile and did not germinate. The development of seed-coats and hairs of these sterile seeds was apparently normal but none germinated even under the best conditions. Perhaps these seeds might have been developed by the stimulation of the pollen tubes, but were not fertilized normally.

Regarding the differences in the success of reciprocal interspecific crosses, W. P. THOMPSON (1930) reported that, as a rule, in cases in which reciprocal interspecific crosses differed in success, the most successful was generally the one in which the species with the larger chromosome number was used as the female parent.

The results of our crossing experiments, presented here, also proved that in reciprocal interspecific crosses in *Gossypium*, better success was obtained with the larger chromosome number in the female parent than with its reciprocal crosses.

Characteristics of the F_1 Plant

The distinguishing characters of the F_1 plant are its vigor, heterosis and its perfect sterility, either by selfing or back crossing. The pollen of the F_1 plant was used on the stigma of several other varieties, but no seeds were produced.

The morphological differences found between F_1 plants and parents are shown in table 2.

TABLE 2

Cross No. 1 (made in 1928), American cotton (*G. hirsutum*) × Asiatic cotton (*G. herbaceum*)

	Stem length (m.)	No. of fruiting branches	No. of vegetative branches	Color of petals	Spot of petals	Flower-stalk habit
F_1-1	2.8	30	6	Intermediate	Intermediate	Erect
F_1-2	2.5	28	5	Intermediate	Intermediate	Erect
Asiatic	1.2	12	2	Yellow	Deep	Curved downward
American	1.6	13	3	Cream	None	Erect

Cross No. 2 (made in 1930), Egyptian cotton, (*G. barbadens*) × Asiatic cotton (*G. herbaceum*)

F_1	3.8	34	6	Yellow	Deep	Erect
Egyptian	2.4	31	5	Yellow	Deep	Erect
Asiatic	1.9	28	6	Yellow	Deep	Curved downward

In both of the crosses the length of the stem was the chiefest character which distinguishes it from its parents, while the type of leaf, color of petals, shape of bracts and length of stamens always showed intermediate features (Pl. VII Fig. 1 a-b and Pl. VIII, Fig. 2).

In general, the spot of the petals also was of intermediate cha acter but the size of spot was so dissimilar even in the same plant that some flowers had the large spot in petals while others had only the very small spot as shown in Pl. VIII, Fig. 3.

Cytological Studies of F_1 Hybrids

Cytological studies of *Gossypium* have been made by John DENHAM, M. A. (1924) who reported that *G. herbaceum*, or Old World cotton, has 13 chromosomes in haploid number while *G. hirsutum* or New World cotton, has 26 in reduced number. No work has yet been reported, however, on cytological features regarding the interspecific

hybridization between *G. herbaceum* and *G. hirsutum* or between *G. herbaceum* and *G. barbadens*.

The cytological investigations of hybrid here reported refer to the crosses between *G. hirsutum* × *G. herbaceum* and *G. barbadens* and *G. herbaceum*.

For fixing the pollen mother-cells BOUIN's solution was used and imbedded in paraffin. The sections were cut 10-15$^{\mu}$ thick and stained with iron-alum haematoxylin. BELLING's aceto-carmine method was also used and the behavior of the chromosomes was then observable more clearly than on permanent slides.

The Chromosome Number of the Parents

The haploid chromosome number of *G. herbaceum* or Manshuzairaimen was counted as 13, while that of *G. hirsutum* or Reihomen and *G. barbadens* or Aschmouni was counted as 26 (Text-figs. 1 a, b, c).(1) No abnormalities were observed in the chromosome behavior in the meiosis of the pollen mother-cells of these parents.

Chromosome Behavior of the First Meiotic Division

In the heterotypic metaphase of the pollen mother-cells of the F_1 hybrid between *G. hirsutum* and *G. herbaceum*, or between *G. barbadens* and *G. herbaceum*, usually 13 bivalent chromosomes were clearly counted on the equatorial plane. The chromosome attachment of each geminus was quite compact and bivalents could clearly be distinguished from univalents according to their size (Text-fig. 2 and 4a). As to the methods of forming gemini we were not be able to determine whether the mating took place between two chromosomes derived from different parents or those derived from one and the same parent, because the chromosomes of both parents were so similar in shape and size that they could not be distinguished from each other.

The arrangement of the chromosomes on the equatorial plane was quite irregular in most cases. Occasionally, however, the bivalents arranged themselves in the central portion of the equatorial plate, and the univalents were located in its periphery.

(1) All text-figures (1–7) are drawn with the aid of ABBE's large camera, ZEISS apochromatic objectives 2 mm., 4 mm. and compensation ocular K. 10.

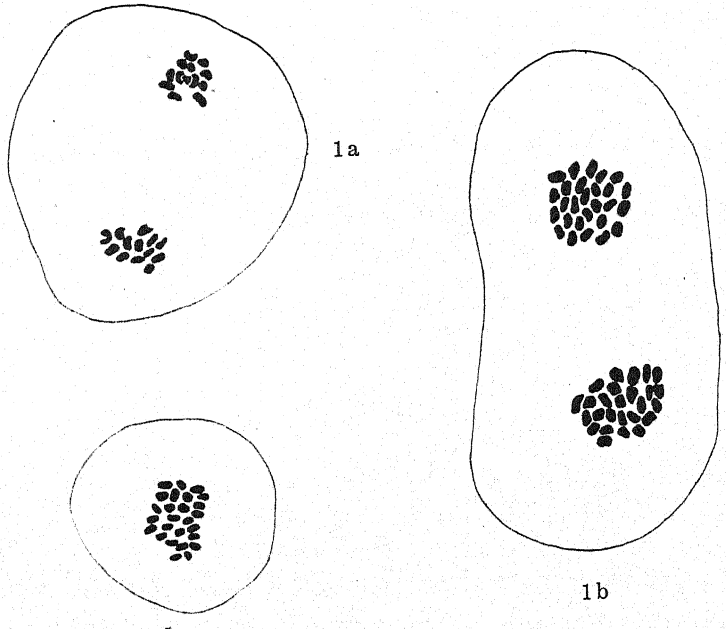


Fig. 1a. *G. herbaceum*, (Manshuzairaimen). Heterotypic anaphase. n = 13. ×900.
b. *G. hirsutum*, (Reihomen). Heterotypic anaphase. n = 26. ×900.
c. *G. barbadens*, (Aschmouni). Heterotypic metaphase, n = 26. ×400.
(All three reduced to 2/3 in reproduction).

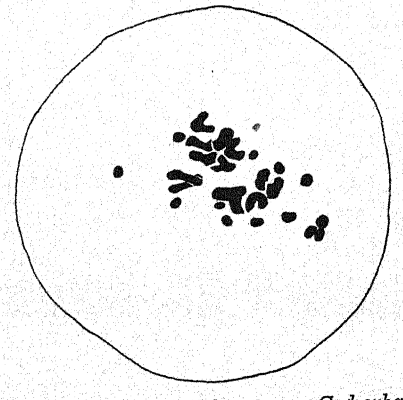


Fig. 2. F_1 of *G. hirsutum* × *G. herbaceum*. Heterotypic metaphase showing 13 bivalents and 13 univalents. ×900.
(Reduced to 2/3 in reproduction).

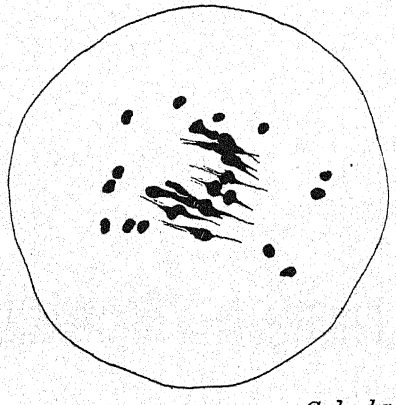
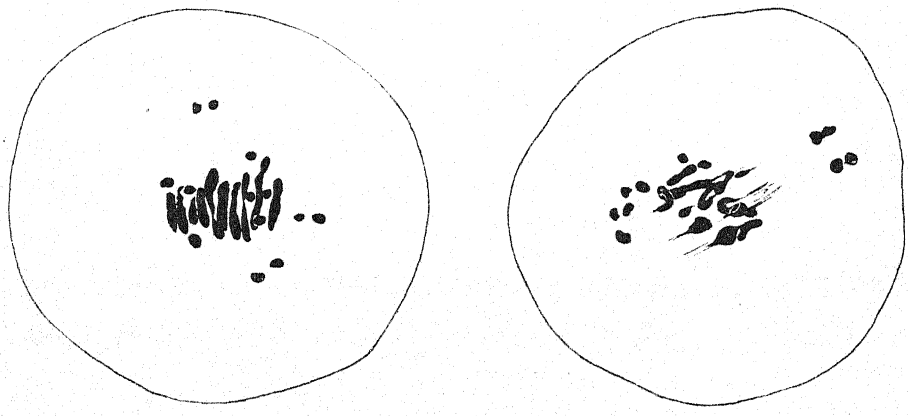


Fig. 3. F_1 of *G. hirsutum* × *G. herbaceum*. Metaphase of heterotypic division: univalents moved irregularly without division. ×900.
(Reduced to 2/3 in reproduction).

In many cells the bivalent chromosomes were divided in the first division and the separated chromosomes moved to each pole as shown in Text-figs. 3 and 4a, but occasionally there was found one or more bivalents located in the cytoplasma without division. The univalent chromosomes moved irregularly without division in the first meiotic division. Some univalents, therefore, were located in the cytoplasma while some were carried to the poles.



4a 4b

Fig. 4a. F_1 of *G. barbadens*×*G. herbaceum*.
Metaphase of heterotypic division. ×900.
b. F_1 of *G. barbadens*×*G. herbaceum*.
Metaphase of heterotypic division. ×900.
(Both reduced to $^2/_3$ in reproduction.)

Chromosome Behavior of the Second Division

The chromosome behavior of the second division of F_1 hybrid was more irregular than in the case of the first meiotic division.

In the homoeotypic metaphase of the pollen mother-cells of the F_1 hybrid, chromosomes were distributed irregularly in the cytoplasma and could rarely be found regulary arranged on the equatorial plane (Text-figs. 5 and 6). In most cells bivalent chromosomes which were not divided in the first division were found even in the second division and two or more groups of chromosomes were located in the cytoplasma. Most of the univalent chromosomes of each group

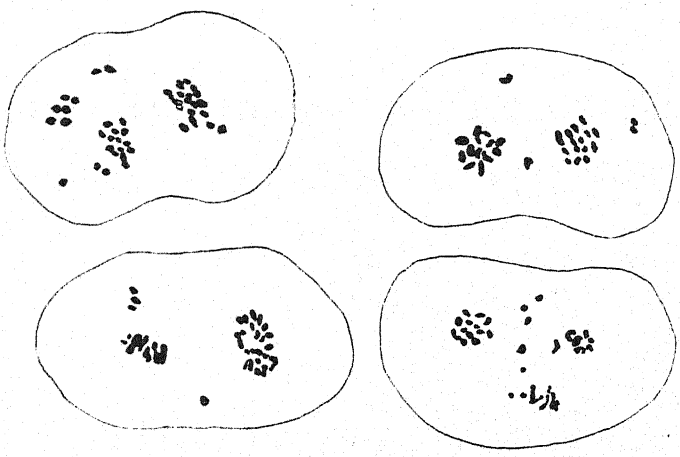


Fig. 5. F_1 of *G. hirsutum* × *G. herbaceum*. Homoeotypic division showing irregular distribution of chromosomes in the cytoplasma. ×400.

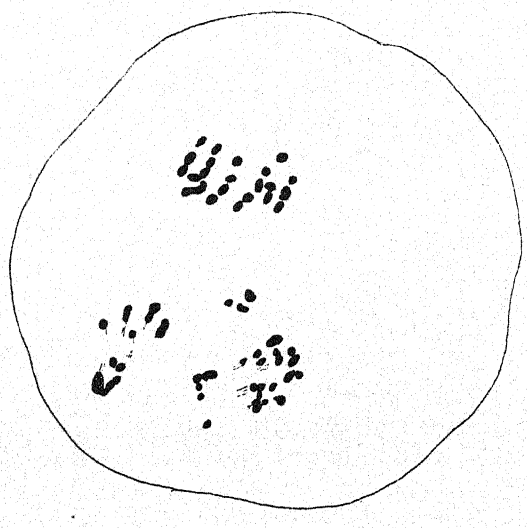


Fig. 6. *G. barbadens* × *G. herbaceum*. Homoeotypic division. ×900.

and bivalent chromosomes were divided in homoeotypic division and the split halves of each chromosomes were separated towards different poles, but some were not divided and left as dyad chromosomes. The pollen formation of the tetrad stage was therefore very abnormal and more than four pollen grains were formed from one pollen mother-cell as shown in Text-fig. 7.

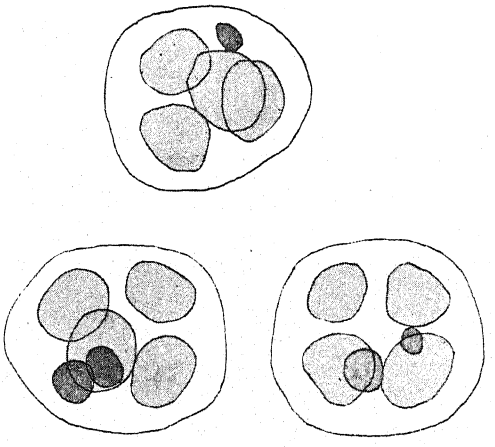


Fig. 7. *G. hirsutum* × *G. herbaceum*. Micro-photograph of abnormal tetrads. ×200.

Table No. 3 shows the variation of the number of pollen grains formed from one pollen mother-cell.

TABLE 3

No. of pollen grains	0	1	2	3	4	5	6	7	8	9	10	11	Total
Frequency				3	4	15	20	19	10	5	4		80

A large number of pollen mother-cells of F_1 plant produced more than four pollen grains and rarely formed three or four pollen grains as shown in Table No. 3. The size of formed pollen grains was therefore quite irregular and perhaps there were many pollen grains which contain an unequal number of chromosomes. Large numbers of pollen grains smaller than those of the parents appeared imperfect under the microscope and if we will judge according to their shape and contents they may be abortive.

It is difficult to say whether the large and apparently perfect pollen grains were abortive or not, although in our crossing experiments unsuccessful results were obtained with them. Further crossing experiments will be necessary to determine whether or not the F_1 hybrids between the Old World and New World cottons will always show perfect sterility.

According to the results of back crosses, the egg cells may be abortive, as in the case of pollen grains, although unfortunately their formation has not yet been studied cytologically. It is clear, however, that the sterility of the F_1 plant in our interspecific hybridization of cotton depends on the abortion of the germ cells.

Summary

1. Interspecific hybridization between Old World and New World cotton has been made. Although no work has been hitherto reported regarding the F_1 hybrid when a New World cotton was used as the female parent in the species hybridization of cotton, we succeeded in obtaining crosses where a New World cotton was used as the female parent.

2. Development of F_1 hybrid was much more vigorous than that of the parents, but perfect sterility was observed in the case of its self-fertilization, and even in that of back crosses or in that of fertilization with pollen of other varieties.

3. Cytological studies of pollen mother-cells of F_1 hybrid were made and it was found that the chromosome behavior in meiotic division was quite irregular.

4. Perfect sterility of F_1 hybrid was found to depend on the formation of abortive germ cells.

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Explanation of Plates VII–VIII

Plate VII

Fig. 1 a. Showing the vigor of the F_1 plant.
F_1 (*G. hirsutum* × *G. herbaceum*)
♀ (*G. hirsutum*) ♂ (*G. herbaceum*)
Photographed on the 20th September.

b. Showing the vigor of the F_1 plant.
F_1 (*G. barbadens* × *G. herbaceum*)
♀ (*G. barbadens*) ♂ (*G. herbaceum*)
Photographed on the 5th July.

Plate VIII

Fig. 2. Showing the shape of bracts and length of stamens of parents and of the hybrid.

Fig. 3. Showing the dissimilar size of spot of petals in the same plant of hybrid between *G. hirsutum* and *G. herbaceum*.



Fig. 1a



Fig. 1b

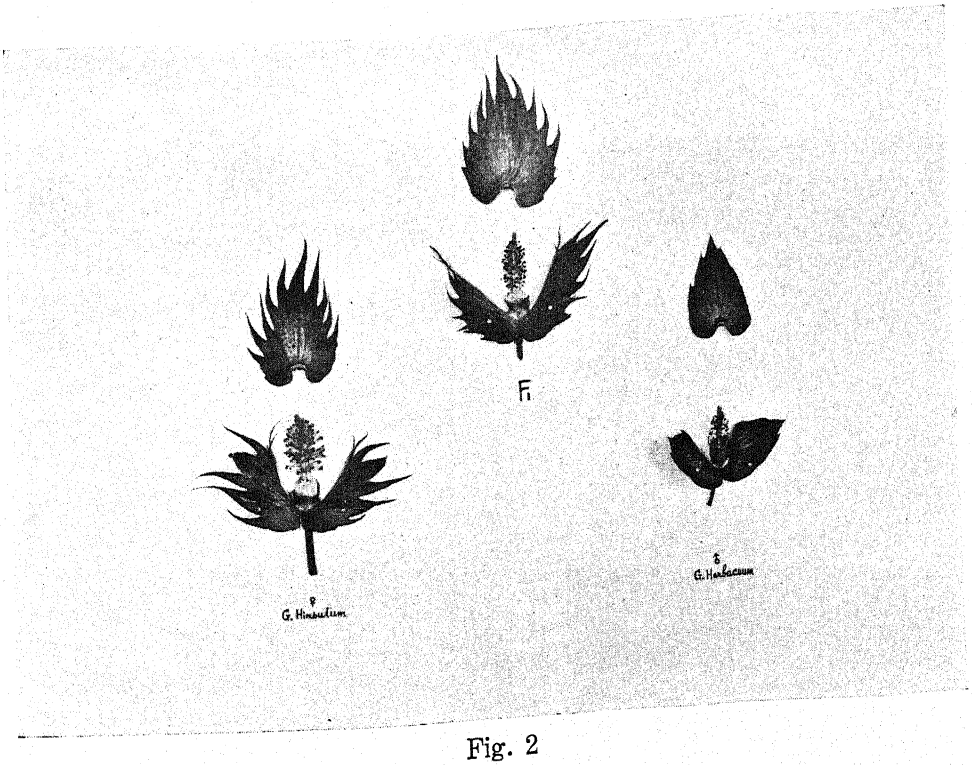


Fig. 2

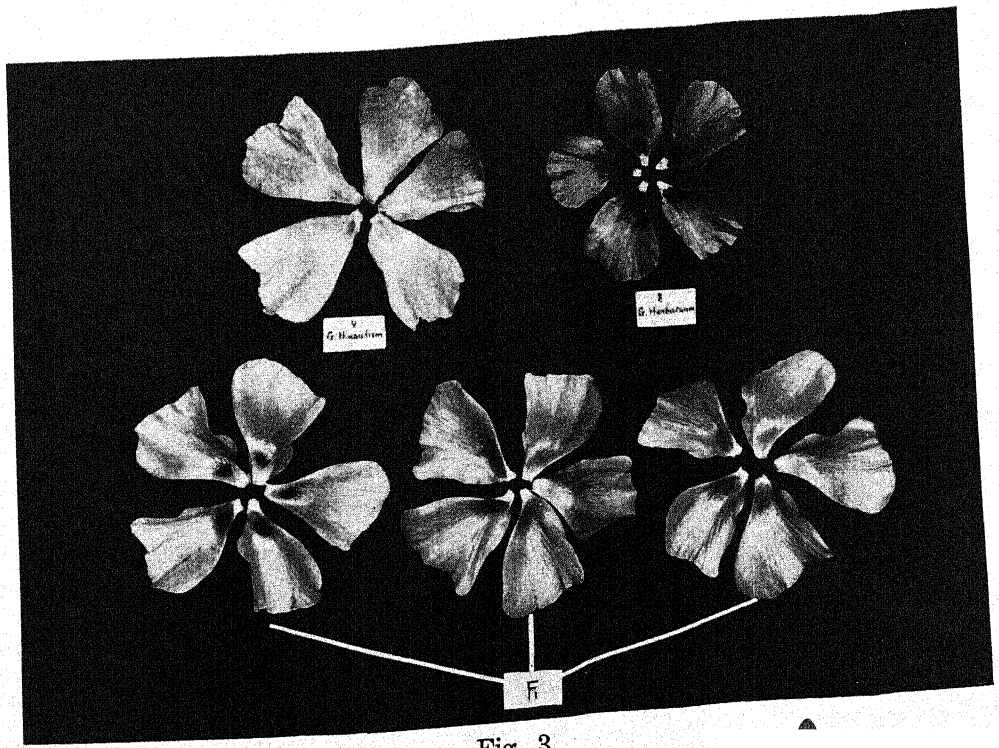


Fig. 3

Cardinal Temperatures of Pea-wilt *Fusaria* in Culture

By Kogo TOGASHI

With One Text-figure

(Received May 14, 1931)

Introduction

In a previous paper (TOGASHI, 1926) the author made a study of three *Fusaria* found associated with pea-wilt in Japan. He identified them, with the assistance of Dr. H. W. WOLLENWEBER, as *Fusarium arthrosporioides* SHERB., *F. sporotrichioides* SHERB., and *F. anguioides* SHERB. In that paper the general cultural characters were described as well as the pathogenicity of these *Fusaria*, often with a comparative study of *F. martii* APP. et WR. var. *minus* SHERB. (*F.martii* APP. et WR. var. *Pisi* JONES) which is reported to cause a stem and root rot of peas in the United States of America (JONES, 1923; JONES and LINFORD 1925).

The present paper sets forth the temperature relations of the *Fusaria* in culture, estimating the cardinals for the mycelial growth and the sporulation.

Experiment I

The cultures were grown in uniform PETRI dishes or ERLENMEYER flasks on potato-decoction agar containing one per cent dextrose. They were kept in incubators set at each of the following temperatures at five degree intervals: 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° C. The increase in the diameter of the mycelial mats was measured daily in two rectangular directions. More than two cultures of each of the fungi were employed for the measurement at each temperature and averaged. The strains used were as follows:—

F. arthrosporioides—single spore strain A-24
F. sporotrichioides—single spore strain B-30

F. anguioides—single spore strain C-33
F. martii var. *minus*—LINFORD's strain from America.

The results of the experiment are presented in Tables 1 and 2, and the average growths on the 7th day are plotted in Fig. 1, a and b.

TABLE 1.

Daily growth of *F. arthrosporioides*, *F. sporotrichioides*, *F. anguioides*, and *F. martii* var. *minus* in potato-dextrose agar at different temperatures (C°). Record for mycelial mats given as averages in cm.

PETRI dishes series

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
				F. arthrosporioides			
5	0	0	0	0	0	0	1.3
10	0	0	0	Visible	0.9	1.2	4.0
15	0.6	1.1	1.9	2.9	3.8	5.2	8.8+
20	1.1	2.6	3.6	4.8	6.7	7.0	8.8+
25	1.6	3.1	3.8	4.1	4.6	5.2	8.8+
30	0.8	1.2	1.7	2.0	2.1	2.6	3.4
35	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
				F. sporotrichioides			
5	0	0	0	0	Visible	Visible	3.1
10	0	Visible	0.9	1.2	1.6	2.4	5.5
15	0.8	1.6	2.7	3.4	4.1	4.5	6.0
20	1.4	3.2	3.6	4.9	5.6	6.8	8.8+
25	2.1	3.4	4.2	5.2	6.8	7.1	8.8+
30	1.6	2.7	3.2	3.8	4.3	5.1	6.3
35	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0

TABLE 1.—(*Continued*)

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
			F. anguioides				
5	0	0	0	0	0	Visible	2.2
10	0	Visible	0.7	0.9	1.4	1.6	3.9
15	0.9	1.3	2.2	2.3	3.1	3.4	6.7
20	1.0	2.3	3.4	4.1	4.8	5.5	8.8+
25	1.6	2.1	2.8	3.0	3.2	3.8	4.5
30	Visible	Visible	1.1	1.5	2.0	2.3	3.7
35	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
			F. martii minus				
5	0	0	0	0	0	0	Visible
10	0	0	0	0	Visible	0.8	3.6
15	Visible	0.9	1.5	2.7	3.3	4.8	6.4
20	0.7	2.7	4.3	5.1	5.6	7.6	8.8+
25	5.0	6.0	8.8	8.8+	8.8+	8.8+	8.8+
30	2.1	3.6	4.6	7.2	8.0	8.3	8.5
35	1.2	2.2	4.5	6.5	7.2	7.8	8.2
40	0	0	0	0	0	0	0

TABLE 2.

Daily growth of *F. arthrosporioides*, *F. sporotrichioides*, and *F. anguioides* in potato-dextrose agar at different temperatures (C°). Record for mycelial mats given as averages in cm.

ERLENMEYER flask series

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
				F. arthrosporioides			
5	0	0	0	0	Visible	Visible	2.0
10	0	0	0	Visible	0.8	1.5	6.3
15	0.6	1.1	1.9	3.1	4.4	5.8	6.5+
20	1.1	2.3	4.1	5.7	6.3	6.5+	6.5+
25	0.6	1.7	2.9	3.7	4.6	5.1	6.5+
30	0.6	1.1	1.4	1.8	2.2	2.7	3.4
35	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
				F. sporotrichioides			
5	0	0	0	0	Visible	Visible	2.7
10	0	0	0.9	1.1	1.6	2.5	6.2
15	0.7	1.3	2.7	3.6	4.8	5.5	6.5+
20	1.2	2.6	4.0	6.5+	6.5+	6.5+	6.5+
25	2.2	4.0	5.0	6.5+	6.5+	6.5+	6.5+
30	2.0	2.8	3.1	6.5	6.5+	6.5+	6.5+
35	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
				F. anguioides			
5	0	0	0	0	Visible	Visible	1.9
10	0	Visible	0.7	1.1	1.7	2.5	5.4
15	0.7	1.3	2.1	4.5	5.5	6.5	6.5+
20	1.4	2.6	4.0	5.6	6.0	6.5+	6.5+
25	1.6	2.4	2.6	2.8	3.1	3.3	5.0
30	Visible	Visible	1.3	1.9	2.4	2.8	4.8
35	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0

F. arthrosporioides. The growth of this fungus was most vigorous at 20° C. both in PETRI dishes and in ERLENMEYER flasks. At 15°, 20°, 25°, and 30° C. the fungus showed a measurable amount of growth 2 days after inoculation. At 10° C. there was no visible growth until the 5th day after inoculation, but it was thereafter fairly rapid. At 5° C. a visible growth was observed in the ERLENMEYER flasks on the 6th day and then it was very slow, 2.0 cm. in average diameter after 14 days. In the PETRI dishes no visible growth occurred on the 7th day and there was only 1.3 cm. in 14 days. At 35° and 40° C. no growth was observed even after 2 weeks' incubation. At any rate the mycelial growth of this fungus seemed to be more rapid in ERLENMEYER flasks than in PETRI dishes, especially under the condition of lower temperatures.

F. sporotrichioides. The fungus grew at temperatures ranging from 5° to 30° C. with an optimum at the higher temperatures of 20°, 25°, and 30° C. At 30° C. the daily growth, as shown by the average diameter of mycelial mats, was less than at 20° and 25° C., but more vigorous with thick floccose aerial mycelium. At 5° C. there was no visible growth until the 6th day and then growth was slow, 3.1 cm. diameter in PETRI dishes and 2.7 cm. in ERLENMEYER flasks in 14 days. At 10°C. considerable growth occurred on the 4th day and the subsequent growth was fairly rapid.

F. anguioides. As also in the case of *F. arthrosporioides* and *F. sporotrichioides* the growth of this fungus took place between 5° and 30° C. The rate of daily growth, however, seemed to be less in comparison with that of the two fungi mentioned above. The best growth was shown at 20° C. in PETRI dishes and at 15° C. in ERLENMEYER flasks. This difference appeared to be due to the moisture relations of the culture. There was a visible growth at 5° C. on the 6th or 7th day, at 10° C. on the 3rd day, and at 30° C. on the 2nd day.

It is of interest to note that at a higher temperature, 30° C., the aerial mycelium was produced very vigorously, that at lower temperatures ranging from 5° to 25° C. the creeping mycelium developed loosely, and that at lower temperatures the characteristic sporulation occurred over the surface of the mycelial area. The following temperatures show the order in sporulation 15°, 20°, 10°, 25°, and 5° C.

F. martii var. *minus.* This fungus seemed to be the most thermophilic among the *Fusaria* studied, growing at 35° C. where the others showed no growth. At 40° C., however, it did not start growth even after 14 days' incubation. At 5° C. it grew very slightly on the 14th

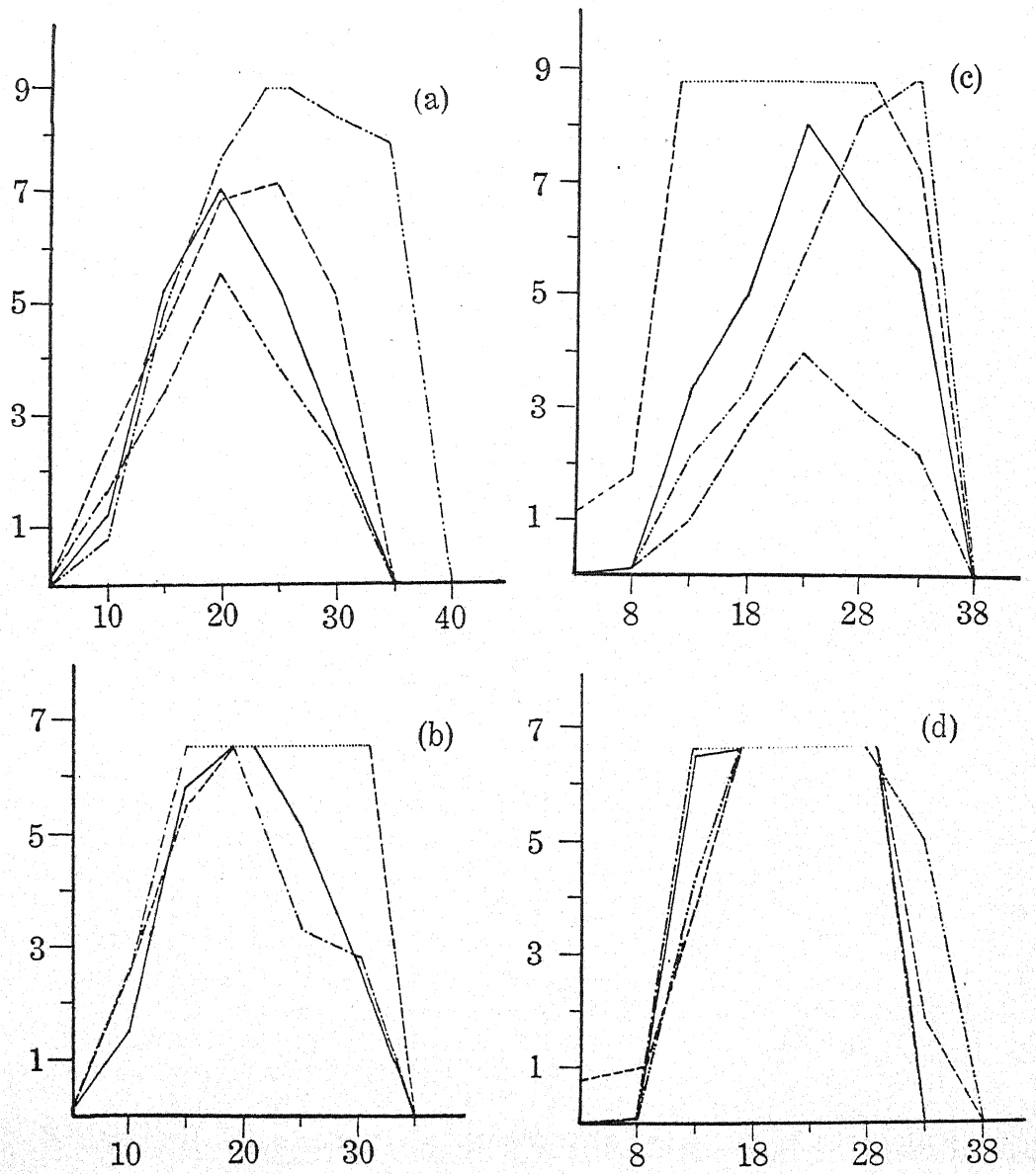


Fig. 1. A graphic representation of the mycelial growth of *Fusaria* at different temperatures on the 7th day. (a) On potato-dextrose agar in PETRI dishes. (b) On the same medium in ERLENMEYER flasks. (c) On pear-decoction agar in PETRI dishes. (d) The same as a, but in ERLENMEYER flasks. Abscissa: temperatures in degrees Centigrade. Coordinate: average diameter of mycelial mats in cm.

———— *F. arthrosporioides.* – – – – – – – – – *F. sporotrichioides.*
—·—·—·— *F. anguioides.* —··—··—·· *F. martii* var. *minus.*

day. The most vigorous growth took place at 30° C., although the average diameter of mycelial mats was smaller at this temperature than at 25° C., sometimes at 20° C. It was characteristic of this fungus that the mycelium spreads loosely over the surface of the medium.

The sporulation occurred at temperatures ranging from 10° to 35° C. with an optimum of 20°–25° C.

Experiment II

The method and the fungi used were the same as in the foregoing experiment, but the cultural media were pear-decoction agar in PETRI dishes and potato-dextrose agar in ERLENMEYER flasks. The temperature was varied by five degrees intervals from 3° to 38° C.

The results are given in Tables 3 and 4, and graphically in Fig. 1, c and d.

TABLE 3.

Mycelial growth of *F. arthrosporioides*, *F. sporotrichioides*, *F. anguioides*, and *F. martii* var. *minus* in relation to various temperatures in PETRI dishes on pear-decoction agar (cm.)

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
			F. arthrosporioides				
3	0	0	0	0	0	0	0.6
8	0	0	0	0	0	Visible	2.6
13	0	Visible	0.9	1.6	2.2	3.2	8.1
18	0	1.0	2.1	2.9	3.7	5.0	8.7+
23	1.2	3.0	4.7	6.0	7.0	8.0	8.7+
28	0.8	2.0	3.4	4.5	5.3	6.6	8.7+
33	Visible	0.9	1.3	3.2	4.8	5.5	8.7
38	0	0	0	0	0	0	0

TABLE 3.—(*Continued*)

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
			F. sporotrichioides				
3	0	0	0	0	0.8	1.1	2.8
8	0	0	Visible	1.1	1.4	1.8	7.5
13	Visible	0.7	1.0	6.5	8.8	8.8+	8.8+
18	1.4	8.6	8.8	8.8	8.8+	8.8+	8.8+
23	3.0	4.5	6.5	7.3	8.0	8.8+	8.8+
28	1.3	8.6	8.6	8.8+	8.8+	8.8+	8.8+
33	0	0.6	1.2	2.2	2.7	7.0	8.8
38	0	0	0	0	0	0	0
			F. anguioides				
3	0	0	0	0	0	0	Visible
8	0	0	0	0	0	Visible	3.8
13	0	0	Visible	Visible	0.8	1.0	5.5
18	0	Visible	1.0	1.4	1.9	2.7	7.7
23	Visible	0.7	1.5	2.2	3.0	4.0	8.7
28	0	Visible	1.3	1.5	2.0	3.0	8.7
33	0	0	0	0.8	1.1	2.2	8.7
38	0	0	0	0	0	0	0
			F. martii minus				
3	0	0	0	0	0	0	0
8	0	0	0	0	0	Visible	1.5
13	0	Visible	1.0	1.4	1.6	2.1	4.2
18	0.7	1.1	1.8	2.4	2.9	3.3	6.9
23	1.8	2.7	3.8	4.6	5.0	5.7	8.8
28	2.4	3.9	5.0	6.1	7.2	8.2	8.8+
33	2.4	3.4	8.6	8.6	8.6	8.8+	8.8+
38	0	0	0	0	0	0	0

TABLE 4.

Mycelial growth of *F. arthrosporioides*, *F. sporotrichioides*, *F. anguioides*, and *F. martii* var. *minus*, in relation to various temperatures in ERLENMEYER flasks on potato-dextrose agar (cm.).

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
			F. arthrosporioides				
3	0	0	0	0	0	0	0.7
8	0	0	0	0	0	Visible	6.0
13	0	Visible	0.8	1.7	2.7	6.5	6.6+
18	1.1	2.5	4.1	6.1	6.6	6.6	6.6+
23	0.9	4.5	6.6+	6.6+	6.6+	6.6+	6.6+
28	0.8	1.8	4.5	4.8	5.5	6.6+	6.6+
33	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0
			F. sporotrichioides				
3	0	0	0	0	Visible	0.8	1.9
8	0	0	Visible	Visible	0.8	1.0	6.6
13	Visible	1.2	1.9	2.6	3.2	3.8	6.6
18	1.5	2.4	3.6	4.6	5.7	6.6+	6.6+
23	2.4	5.2	6.6	6.6+	6.6+	6.6+	6.6+
28	1.9	4.0	5.0	6.6	6.6	6.6+	6.6+
33	0	0.7	0.9	1.2	1.6	1.8	2.8
38	0	0	0	0	0	0	0

TABLE 4.—(*Continued*)

Temp.	2nd day	3rd day	4th day	5th day	6th day	7th day	14th day
			F. anguioides				
3	0	0	0	0	0	0	0
8	0	0	0	0	0	Visible	6.5
13	0	0	Visible	0.9	6.5	6.5+	6.5+
18	0	1.2	6.5	6.5+	6.5+	6.5+	6.5+
23	Visible	1.4	2.7	3.9	5.5	6.5+	6.5+
28	0	4.5	5.7	6.5	6.5+	6.5+	6.5+
33	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0
			F. martii minus				
3	0	0	0	0	0	0	0
8	0	0	0	0	0	Visible	1.0
13	0	Visible	1.2	1.4	1.6	4.3	6.0
18	1.7	4.3	5.3	6.0	6.6+	6.6+	6.6+
23	1.6	2.4	3.7	4.8	5.7	6.6+	6.6+
28	1.6	2.9	3.8	5.5	5.8	6.6	6.6+
33	1.8	2.3	4.4	4.6	4.7	5.0	6.2
38	0	0	0	0	0	0	0

F. arthrosporioides. The fungus grew most vigorously at 23° and 18°C. where the staling in culture took place in 14 days after incubation. The aerial mycelium was produced well at temperatures lying between 13° and 28° C. At 3° C. there was no growth during the period of the first 7 days, and but very scant- at the end of two weeks. The cultures at 8° and 13° C. showed visible growth on the 7th day and the 3rd day respectively. At 33° C. the fungus on pear-decoction agar started to grow within 2 days, continuing a good rate of increase, but no sign of growth could be observed on potato-dextrose agar even after 14 days.

F. sporotrichioides. The fungus grew at temperatures ranging from 3° to 33° C. with some difference in rate of daily growth and in sporulation according to the difference of cultural methods. The sporulation took place between 8° and 33° C. on pear-decoction agar in PETRI dishes, and between 8° and 28° C. on potato-dextrose agar in ERLENMEYER flasks. The optimum temperature for the sporulation seemed to be 18°–28° C. in the former case and 23°–28° C. in the latter. In both cases the fungus showed the most vigorous mycelial growth at 18°–28° C. At these temperatures, especially at 18° and 23° C. the aerial mycelium developed abundantly, and at temperatures higher than 23° C. the cultures showed a tendency for the creeping mycelium to spread somewhat loosely over the agar plates. At 3° C. there was no growth until the 6th day, and then it was very slow, although at the optimum temperature considerable increase was observed on the 2nd day after incubation. At 8°, 13°, and 33° C. the growth started on the 4th day, the 2nd day, and the 3rd day, respectively.

F. anguioides. The growth of this fungus was slow at each temperature, especially on pear-decoction agar. Even at the optimum temperature, 18°–28° C., visible growth was not observed until the 2nd or 3rd day after incubation. The cultures on pear-decoction agar at 33° C. where no growth occurred on potato-dextrose agar, produced creeping mycelium loosely. On the other hand the fungus cultured at 18°–28° C. developed flocculent aerial mycelium abundantly in both media. The sporulation took place in the cultures on potato-dextrose agar at 8° and 23° C., but not in those on pear-decoction agar. At 3° C. very slight growth occurred on pear-decoction agar after 2 weeks' incubation.

F. martii var. *minus.* The fungus grew at temperatures ranging from 8° to 33° C. with an optimum of 18°–33° C. At 8° it started to grow on the 7th day and then continued very slow growth. However, at the higher extreme of temperature, 33° C. considerable amounts of growth were already found on the 2nd day, and then the fungus showed inherently fast growth.

The sporulation took place at temperatures varying from 13° to 28° C., the optimum lying between 13° and 23° C.

TABLE 5.

Cardinal temperatures for mycelial growth of *Fusaria*, determined by various investigators. Degrees in Centigrade. The "/" sign on the left denotes below the temperature, the "/" sign on the right denotes above the temperature, and the signs on the both sides denote about the temperature.

Fungus	Min,	Opt.	Max.	Investigator
F. avenaceum (Fr.) Sacc. Form 1	/7	22	32/	Tu, 1929
Form 2	/7	27	32/	,,
,,	10–16	28–32		Abe, 1928
F. Celosiae Abe		/26/		Tims, Walker, 1924
F. Cepae Hanzawa	7	25–27	35	Tisdale, 1923
F. conglutinans Wr.	9	24–27	33/	Stewart, 1931
F. conglutinans Wr. var. *Betae* St.		/28/		Sideris, 1929
F. cromyophthoron Sid.	4	24–28	32	Simmonds, 1928
F. culmorum (W. G. Sm.) Sacc.	/7	27	32/	Tu, 1929
,, (3 forms & 1 mutant)			36	Haskell, 1919
F. eumartii Carp.		10–15		Horne, 1930
F. fructigenum Fr.	3	25–27	33	Dickson, 1921
F. graminearum Schwabe (*Gibberella Saubinetti* (Mont.) Sacc.)	4	24–29	34	Jones, Johns, Dickson, 1926
,,	/7	27	32/	Tu, 1929
,, (3 forms)	10–11	26–28	34–37	Tisdale, 1917
F. Lini Bolley		24–28		Jones, Tisdale, 1922
,,	/7	18–30		Broadfoot, 1926
,,	10	28.5–30		Tochinai, 1926
,,	9–10	28	37	Clayton, 1920, 1923
F. lycopersici (Sacc.) Wr.				
F. martii AP. et WR. var. *minus* SHERB.	5–6	20–34		Jones, 1933
F. martii Ap. et Wr. var. *Phaseoli* Burkh.	/12–13	30.5–31.5	/38.5–39	Reddick, 1917
F. nivale (Fr.) Ces.	/7	22	/32	Tu, 1929
(*Calonectoria graminicola* (B. et B.) Wr.)	8/	24–32	/35	Porter, 1928
F. niveum E.F.S.	5	15–30	/37.5/	Smith, Swingle, 1904
F. oxysporum Schlecht		/30/	38–40	Link, 1916
,,		26–32	/40/	Haskell, 1919
,,				
F. oxysporum S. var. *Gladioli* Mass.	5	27.5	35	Massey, 1926
F. oxysporum S. var. *medicaginis* W.	/3	25	37 or 38	Weimer, 1930
F. Solani Sacc.	/7	32	32/	Tu, 1929
F. vasinfectum Atk.	/10	28–30	/38/	Neal, 1927
Average	7.1–7.5	25–27.7	34.8–35	

TABLE 6.

Cardinal temperatures for the mycelial growth and sporulation of *Fusaria*, determined by the writer in the present experiments. Degrees in Centigrade.

Fungus	Min.	Opt.	Max.
Mycelial growth			
F. arthrosporioides	3	15–25	33/
F. sporotrichioides	/3	18–30	33/
F. anguioides	3	15–28	33/
F. martii var. *minus*	5	18–33	35/
Sporulation			
F. arthrosporioides	—	—	—
F. sporotrichioides	8	18–28	33
F. anguioides	5	8–20	25
F. martii var. *minus*	10	13–25	35

Conclusion

A large amount of work from various sides has been reported by various investigators on temperature relationships of *Fusaria*. Available reports relating to the cardinal temperatures for mycelial growth in culture, are represented in a summarized form in Table 5. A summary of the present experiments is also given in Table 6. From comparing the data in the tables conclusions may be drawn as follows :—

1. Among *Fusaria* studied *F. martii* var. *minus* was the most thermophilic with a growth range of 5° to above 35° C., *F. sporotrichioides* was the next, *F. anguioides* came after, and *F. arthrosporioides* the last.

2. Minimum temperatures for the growth of *F. arthrosporioides*, *F. sporotrichioides*, *F. anguioides*, and *F. martii* var. *minus* were

respectively 3°, below 3°, 3°, and 5° C. On the whole, as one may recognize from Table 5, the minimum temperatures for the growth of each of the *Fusaria* cited are much higher than 3° C. Exceptions are *F. graminearum* (the conidial stage of *Gibberella Saubinetii*, 3° C.) and *F. oxysporum* var. *medicaginis* (below 3° C.).

3. There was no significant difference between the maximum temperatures for the growth of *F. arthrosporioides*, *F. sporotrichioides*, and *F. anguioides* under our experimental conditions, each showing above 33° C. But the maximum of *F. martii* var. *minus* was somewhat higher being 35° C. Although many exceptions can be mentioned from Table 5, *F. arthrosporioides*, *F. sporotrichioides*, and *F. anguioides* took generally a lower maximum temperature than that of all the other *Fusaria* cited, but *F. martii* var. *minus* showed a similar gradient to the average maximum.

4. The *Fusaria* studied grew vigorously at comparatively a wide range of temperature. Vigorous growth of *F. martii* var. *minus* and *F. sporotrichioides*, however, occurred at higher temperatures than *F. arthrosporioides* and *F. anguioides*.

5. As seen in most fungi the temperature limits for the sporulation of the *Fusaria* studied were narrower than those for the mycelial growth. It was a noticeable evidence that the minimum, optimum and maximum temperatures for the sporulation of *F. anguioides* were much lower than those of *F. sporotrichioides* and *F. martii* var. *minus*, and also that the sporulation of *F. martii* var. *minus* took place most abundantly at lower temperatures than *F. sporotrichioides*. No sporulation occurred in any of cultures of *F. arthrosporioides* during the period of the present experiments.

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Über die Beeinflussung des Wachstums der Schimmelpilze durch die von Rosahefen gebildeten Stoffe

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Hierzu Tafel IX und 6 Textfiguren

(Eingegangen am 17. Juni 1931)

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Einleitung

Im Gegensatz zu der Tatsache, dass verschiedene Bakterien und Hefen für ihr Leben das Vorhandensein gewisser organischen Stoffe, die man im allgemeinen als Vitamin oder Bios bezeichnet, oft unbedingt nötig haben, ist es seit früher bekannt, dass die Schimmelpilze wie *Aspergillus* für das Wachstum solche Stoffe durchaus entbehren können. Manche Forscher[1] haben aber konstatiert, dass das Wachstum solcher, auf rein synthetische Medien züchtbaren Organismen auch durch Zusatz sehr geringfügiger Menge der durch andere lebende Zellen gebildeten Stoffe erheblich gesteigert wird. Solchen wachstumsbeschleunigenden Stoffen gegenüber liegen uns andererseits auch verschiedene Stoffe vor, die gleichfalls von gewissen lebenden Zellen gebildet sind und zwar auch in äusserst geringer Menge auf das Leben anderer Organismen stark giftig und oft sogar tötend einwirken. Abgesehen von der wohl bekannten Giftwirkung verschiedener Toxinen oder gewisser Alkaloiden auf höhere Tiere oder Pflanzen sind uns auch mehrere, chemisch noch nicht definierbare Stoffe bekannt,[2] die schon in ganz kleiner Menge den Betriebs- oder Aufbaustoffwechsel der niederen Organismen sichtlich beeinträchtigen. Merkwürdigerweise gibt es nicht selten die Fälle, bei denen die von einem gewissen Mikroorganismus gebildeten Stoffe nicht nur auf andere Organismen, sondern auch auf den betreffenden Organismus selbst giftig wirken. So hat z. B. HAYDUCK[3] festgestellt, dass der Extrakt einer Hefe, wenn man denselben in gewisser Menge zu der normalen Kultur der betreffenden Hefe zusetzt, nicht nur das Wachstum sondern auch die Gärfähigkeit derselben wohl um 80% aufhielt. Eingehendere Untersuchungen von FERNBACH und VULQUIN[4] hat auch ergeben, dass die Giftsubstanz im Extrakt der Hefezellen nicht nur auf Hefen selbst, sondern auch auf das Wachstum verschiedener Bakterien stark hemmend wirkt.

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(1) J. NIKITINSKY: Jahrb. f. wiss. Bot., **40**, 1904, 1.
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(2) C. A. PRATT: Ann. of Bot., **38**, 1924, 564 & 595.
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(3) F. HAYDUCK: Wochenschr. f. Brauer., **26**, 177, 189, u. 201.

(4) A. FERNBACH: Compt. rend. Acad. Sci., 1909, 144.
A. FERNBACH u. E. VULQUIN; Ebenda., 1909, 67.

Die in nachfolgenden Zeilen beschriebenen Versuchsergebnisse geben einen Beispiel dafür, dass das Wachstum der Schimmelpilze durch Zusatz einer ganz kleinen Menge der in verschiedenen Hefezellen enthaltenen Stoffe entweder sehr fördernd oder aber sehr hemmend beeinflusst wird. Unsere besondere Aufmerksamkeit verdient wohl die Tatsache, dass die in gewissen Rosahefezellen enthaltene Substanz, deren chemische Natur noch nicht näher feststellbar ist, in sehr eigentümlicher Weise das Wachstum verschiedener Schimmelpilze, nicht aber das der Hefe beeinträchtigt.

Methodisches

Als Versuchsobjekte für die vorliegende Arbeit kamen folgende Schimmelpilz- und Hefearten in Betracht.

Schimmelpilze		Hefearten
Aspergillus	*aureus*	*Debaryomyces tyrocola*
,,	*Awamori*	*Saccharomyces cerevisiae*
,,	*giganteus*	,, *saké*
,,	*gymnosardae*	,, *apiculatus*(1) (*Pseudosaccharomyces apiculatus*)
,,	*niger*	*Torula* sp.(1)
,,	*ochraceus*	Wildhefe
,,	*Oniki*	Rosahefe (Stammnummer 1–14) (*Torula Suganii, infirmo-miniata, miniata, decolans, koishikawensis, Shibatana Pseudomonilia rubicundula*)(2)
,,	*oryzae*	
,,	*soya*	
Monascus	*purpureus*	*Torula rubra*(3)
Rhizopus	*nigricans*(1)	,, *sanguinea*(3)

Zur Züchtung der Schimmelpilze habe ich mich hauptsächlich folgende Kulturlösung bedient.

(1) An dieser Stelle möchte ich Herren Dr. F. OANA und Dr. K. MATSUMOTO, im Forschungsinstitut für Brauwesen in Takinogawa (Tokyo), für ihre freundliche Zusendung von diesen Stämmen herzlich danken.

(2) Diese neuen Arten von Rosahefen wurden in Japanese Journal of Botany, Vol. **5**, 1931, S. 285, eingehend beschrieben.

(3) Diese Arten sind von K. SAITO in Japanese Journal of Botany, Vol. **1**, 1923, 1, angegeben. Hierbei bin ich Herrn Prof. H. NAGANISHI sehr verpflichtet für die Übersendung dieser beiden Stämme.

Rohrzucker (M/2)	50 ccm	mit doppeltdestilliertem Wasser auf 100 ccm gebracht.
Lösung A[1]	15 „	
KH_2PO_4 (M/2)	15 „	

Als Kulturgefässe dienten ERLENMEYERsche Kolben aus Normalglas von 50 ccm Inhalt, die vor Gebrauch sorgfältig mit Chromschwefelsäuregemisch und dann wiederholt mit doppeltdest. Wasser gereinigt wurden. Die mit Wattepfropfen versehenen Kolben wurden mit je 25 ccm von Kulturlösung beschickt und im Dampftopf 1 Stunde lang sterilisiert. Die Impfung der Kolben wurde mit der Konidiensuspension nach der Vorschrift TAMIYAS[2] ausgeführt.

Um die Beeinflussung des Schimmelpilzwachstums durch Hefezellenzusatz zu prüfen, wurden dieser Kulturlösung die Hefezellen in verschiedener Menge beigefügt, entweder vor oder nach der Impfung der Schimmelpilzkonidien. Die Kultur wurde in einem Thermostat bei 30° oder 27° angestellt. Das Wachstum der Myzelien wurde durch Messung des Trockengewichtes (getrocknet bei 70°) ermittelt.

Beeinflussung des Wachstums von Pilzen durch die abgetöteten Hefezellen

Versuch I

Wir wollen zunächst sehen, ob und wie der Zusatz der abgetöteten Hefezellen das Wachstum verschiedener Schimmel- und Hefepilze beeinflusst. Die Hefen, deren tote Zellen auf ihre Wirkung auf andere Mikroorganismen untersucht werden sollten, wurden zunächst auf Schrägagarboden mit folgender Zusammensetzung kultiviert.

Rohrzucker	8 g	mit destilliertem Wasser auf 100 ccm gebracht.
Pepton	1 „	
Lösung A	2.5 ccm	
KH_2PO_4 (M/2)	2.5 „	
Agar	2 g	

Nach etwa 2 Wochen langer Kultur bei 20° wurde die Hefemasse mit einer dicken Platinöse gesammelt und in sterilem Wasser suspen-

(1) Lösung A enthielt folgende Salze in 100 ccm: NH_4NO_3 4 g, $MgSO_4$ 1 g, $FeCl_3$ (5%) 1 Tropf.

(2) H. TAMIYA: Acta Phytochimica, **3**, 1927, 58.

diert. Um die Hefezellen abzutöten, wurde diese Hefesuspension 30 Minuten lang bei 65° erwärmt. Eine Kulturprobe ergab, dass die Wachstumsfähigkeit der Hefezellen durch diese Behandlung schon gänzlich sistiert ist. Die so erhaltene Hefesuspension wurde ferner sorgfältig verdünnt, bis sie etwa 100 Millionen Zellen pro 1 ccm enthielt. Diese Hefesuspension wurde dann den Kulturlösungen anderer Schimmelpilze und Hefen zugefügt, wobei die Kulturlösung folgende Zusammensetzung zeigten.

Rohrzucker (M/2)	50 ccm
Lösung A	15 ,,
KH_2PO_4 (M/2)	15 ,,
Hefesuspension	20 ,,
Summe	100 ,,

Zunächst wurde der Einfluss der abgetöteten Zellen von zwei Hefearten, nämlich der Bierhefe und einer Rosahefe,[1] auf verschiedene *Aspergillus*-Arten, *Monascus purpureus*, *Rhizopus nigricans* und auch auf verschiedene Hefearten untersucht.

Um den Grad des Wachstums anschaulich zu machen, habe ich bei Schimmelpilzen den Prozentsatz des Trockengewichtes zu demjenigen der Kontrollkultur (ermittelt stets aus drei Parallelversuchen) angegeben, während bei Hefen folgender Zahlenwert als Massstab der Wachstumsgrösse angewandt wurde.

$$\text{Vermehrungsgrad} = 100 \times \frac{(\text{Zahl der gewachsenen Zellen}) - (\text{Zahl der geimpften Zellen})}{(\text{Zahl der geimpften Zellen})}$$

Die durchschnittliche Zellenzahl wurde stets mittels der THOMA-Kammer bestimmt.

Aus der Tabelle I geht ganz deutlich hervor, dass der Zusatz von abgetöteten Hefezellen das Wachstum von Schimmelpilzen und Hefen in ganz verschiedener Weise beeinflusst, und zwar, dass die Rosahefe und die Bierhefe auf das Gedeihen von Schimmelpilzen ganz unterschiedlichen Einfluss ausüben. Der Zusatz der Rosahefezellen wirkt nämlich immer mehr oder minder hemmend auf das Wachstum von allen untersuchten Schimmelpilzen, während derselbe bei verschiedenen

(1) Rosahefe Stamm-Nr. 1, d.h. *Torula Suganii*. (Siehe Japanese Journ. of Bot., **5**, 1931, 285.)

TABELLE I.

		Kontrolle	Bierhefezusatz	Rosahefezusatz
Aspergillus	*niger*	100.0	103.7	12.4
,,	*aureus*	,,	135.5	27.4
,,	*Awamori*	,,	131.9	37.2
,,	*Oniki*	,,		42.3
,,	*giganteus*	,,	194.7	60.4
,,	*oryzae*	,,		61.8
,,	*soya*	,,	137.7	64.9
,,	*ochraceus*	,,	121.7	64.9
,,	*gymnosardae*	,,	112.1	65.3
Monascus	*purpureus*	,,		61.8
Rhizopus	*nigricans*	,,		83.1
Saccharomyces	*cerevisiae*	,,	209.8	163.1
,,	*saké*	,,	401.3	198.3
Debaryomyces	*tyrocola*	,,		469.7
Torula	*Suganii*	,,		137.2

Kulturdauer: 4 Tage. *Aspergillus*-Arten bei 30°; andere Pilz- und Hefe-Arten bei 25°.

Hefearten stets sogar eine Förderung des Wachstums hervorruft. Im Gegensatz zu der Rosahefe, bewirkt die Bierhefe sowohl auf Schimmelpilze als auch auf Hefen immer eine mehr oder minder deutliche Begünstigung des Wachstums. Die deutliche Verzögerung des Wachstums von *Aspergillus oryzae* bei Rosahefenzusatz lässt sich dadurch zeigen, dass dabei die Pilzmyzelien, ohne vollkommene Decke zu bilden, stets entlang der Gefässwände ringförmig wachsen, während bei Kontroll- sowie Bierhefenzusatz-Kultur immer eine vollkommene Decke gebildet wird. Ferner findet man bei Rosahefezusatz eine Menge der in der Kulturlösung gesunkenen Fetzen der dünnen Hyphen (siehe Taf. IX, Fig. 1). Das Wachstum von *Monascus purpureus* wird durch Zusatz der Rosahefezellen sichtlich verzögert. Während die Lösung der Kontrollkultur durch die vom Pilz ausgeschiedenen Farbstoffe dunkelrot gefärbt ist, zeigt die durch Rosahefezusatz vergiftete Kultur, entsprechend dem sehr schwachen Pilzwachstum, fast gar keine Färbung. Die Giftwirkung der Rosahefezellen tritt bei *Rhizopus*

nigricans nur im früheren Stadium des Wachstums deutlich zu Tage, wie es aus Taf. IX, Fig. 2 hervorgeht.

Versuch II

Die Tatsache, dass die auf Schimmelpilze, insbesondere *Aspergillus*, giftig wirkende Substanz nur in der untersuchten Rosahefe, nicht aber in Bierhefe enthalten ist, lässt uns zunächst vermuten, dass die in Frage kommende Giftsubstanz in irgend einer Beziehung zur Farbstoffproduktion der Rosahefe stehe. In diesem Versuche habe ich also folgende Stämme der Rosahefen sowie einiger farblosen Hefen auf ihre Wirkung auf das Wachstum von *Aspergillus oryzae* vergleichend untersucht.

Saccharomyces cerevisiae	Wildhefe (farblos)
„ *saké*	Rosahefe Stammnummer 1–14(1)
„ *apiculatus*	*Torula rubra* (SAITO)
Debaryomyces tyrocola	„ *sanguinea* (SAITO)
Torula sp. (farblos)	

Von jedem Hefestamm wurde eine Hefesuspension, die etwa 15 Millionen Zellen pro 1 ccm enthielt, zubereitet. Nach 30 Minuten langer Erwärmung bei 65° wurden 20 ccm von dieser Hefesuspension mit Kulturlösung auf 100 ccm gebracht. Als Kontrolle wurden die Versuchsreihen angestellt, die statt der oben angegebenen Hefesuspensionen dopp ltdestilliertes Wasser enthielten. Die erhaltenen Resultate sind in den Tabellen II u. III zusammengestellt.

Aus Tabelle II und III ersieht man, dass die Giftwirkung auf *Aspergillus*wachstum nicht allen Rosahefenstämmen zukommt, sondern dass bei einigen Stämmen sogar eine ganz deutliche wachstumsbegünstigende Wirkung wie bei allen farblosen Hefen konstatiert wurde.

Jedenfalls kann man aber wohl darauf schliessen, dass die Giftwirkung der untersuchten Rosahefezellen in keiner direkten Beziehung zu ihrem Farbstoffgehalt steht. Zwischen den auf das Pilzwachstum hemmend wirkenden und den beschleunigend wirkenden Rosahefestämmen konnte ich vorläufig nur den Unterschied bemerken, dass die Kolonien der ersteren immer etwas schleimiger als die der letzteren sind.

In folgenden werde ich über die physikalisch-chemischen Eigenschaften und die physiologischen Verhalten der Giftsubstanz, die im Rosahefestamm Nr. 1, *Torula Suganii*, enthalten ist, näher eingehen.

(1) Siehe meine frühere Arbeit in Japanese Journal of Botany, **5**, 1931, 288 ff.

TABELLE II.

Zusatz	Pilzerntegew. (mg)	Verhältn.	Zusatz	Pilzerntegew. (mg)	Verhältn.	Zusatz	Pilzerntegew. (mg)	Verhältn.
Kontrolle	115.0 100.5 112.5	100.0	Rosahefe Nr. 7	119.0 99.5 100.0	97.1	*Sacchar. saké*	176.5 126.0 192.5	150.9
Rosahefe Nr. 1(1)	66.5 58.5 58.0	55.8	Rosahefe Nr. 8	171.5 179.0 144.0	150.8	*Sacchar. cerevis.*	186.0 123.0 192.0	152.8
Rosahefe Nr. 2(1)	45.0 69.5 38.0	46.5	Rosahefe Nr. 9	224.0 221.0 271.0	218.9	*Sacchar. apicul.*	255.0 249.0 285.0	271.0
Rosahefe Nr. 3(1)	65.5 67.0 68.0	61.1	Rosahefe Nr. 10	145.0 127.5 131.0	123.3	*Debaryom. tyrocola*	147.0 139.0 154.5	134.5
Rosahefe Nr. 4(1)	68.5 66.0 63.0	60.2	Rosahefe Nr. 11	153.5 166.0 143.5	141.2	*Torula* (farblos)	203.5 174.0 185.0	171.5
Rosahefe Nr. 5(1)	69.5 84.0 86.5	73.2	Rosahefe Nr. 12	187.0 191.0 155.0	162.3	Wildhefe (farblos)	170.5 165.0 223.5	170.5
Rosahefe Nr. 6	185.0 136.5 144.0	141.9	Rosahefe Nr. 13	132.5 155.0 151.0	133.7			

Kulturdauer: 4 Tage bei 30°. Anfangs-pH 4.0.

TABELLE III.

	Kontrolle (mg)	Rosahefe Nr. 1 (mg)	*T. sanguinea* (mg)	*T. rubra* (mg)
Anfangs-pH	4.0			
Pilzerntegewicht	175.0 205.0 174.0	63.0 89.0 66.0	190.5 184.5 213.5	196.0 183.0 197.5
Verhältnis	100.0	40.2	115.2	104.0

Kulturdauer: 4 Tage bei 30°.

(1) Die Rosahefestämme Nr. 1–5 wurden als *Torula Suganii* zusammengefasst. Vergl. Jap. Journ. Bot., **5**, 1931, 318.

Eigenschaften der das Pilzwachstum affizierenden Substanzen

(1) Einfluss der Temperatur

Versuch III

Um die chemisch-physikalische Natur der in Rosahefezellen enthaltenen, auf das Schimmelpilzwachstum reizend oder vergiftend wirkenden Stoffe näher kennen zu lernen, habe ich zunächst den Temperatureinfluss auf dieselben untersucht. Mit der wirksamsten Rosahefe und der gewöhnlichen Bierhefe wurden wie vorher Zellensuspensionen zubereitet, welche folgender Behandlung unterworfen wurden.

1. Ohne Erwärmung.
2. Bei 65° 30 Minuten lang erhitzt.
3. Bei 100° 1 Stunde lang gekocht.
4. Bei 120-130° unter 1.5 Atm. 30 Minuten lang erhitzt.

Die Suspensionen wurden dann in gleichem Mengenverhältnis wie vorher der gewöhnlichen Kulturlösung zugesetzt; dann wurden diese Kulturlösungen mit den Konidien von *Aspergillus oryzae* und *Asp. niger* geimpft. Das Pilzerntegewicht (in mg) wurde nach 4 Tage langer Kultur bei 30° bestimmt.

TABELLE IV.

Pilz	Zugesetzte Hefe	Versuchsreihe			
		1	2	3	4
Asp. niger	Ohne Zusatz	100.0			
	Bierhefe	71.7	113.3	142.2	159.9
	Rosahefe Nr. 1	43.0	34.5	36.6	30.1
Asp. oryzae	Ohne Zusatz	100.0			
	Bierhefe	59.3	105.8	116.2	112.1
	Rosahefe Nr. 1.	75.3	74.7	75.0	70.8

(1) Durch diese Wärmebeständigkeit unterscheidet sich diese Hemmungssubstanz von derjenigen der sogenannten „Staling". (Siehe PRATT, loc. cit.)

Es zeigte sich aus dieser Tabelle, dass der Zusatz von Rosahefezellen immer, und zwar auch wenn die Hefezellen vorher auf 120-130° erhitzt worden sind, eine ganz deutliche Wachstumshemmung bewirkt, indem das Wachstum von *Aspergillus niger* wohl um 60-70% und dasjenige von *Aspergillus oryzae* um 25-30% herabgesetzt wurde. Der Versuch zeigt ferner, dass das Wachstum der *Aspergillus*-Arten immer bei Zusatz der vorher abgetöteten Bierhefezellen beschleunigt wird, während bei Zusatz von lebenden Zellen stets eine deutliche Störung des Myzelwachstums beobachtet wird.

Gestützt auf die oben angegebene Tatsache kann man darauf schliessen, dass der wachstumshemmende Stoff in Rosahefezellen sowie auch der wachstumsbeschleunigende Stoff in Bierhefezellen in weiter Grenze hitzebeständig sind.

(2) Einfluss des Lichtes

Versuch IV

Es wäre vom Interesse zu erfahren, ob die in Frage kommenden Stoffe durch Belichtung mit ultravioletten Strahlen in ihrer wachstumshemmenden bzw. -beschleunigenden Wirkung auf Schimmelpilz irgendwie modifiziert werden. Es kamen die Rosahefesuspension, der Alkoholextrakt von Rosahefezellen und das Filtrat der Rosahefekultur durch SEITZ-Filter, die in Quarzröhren mit der Quecksilberbogenlampe NAGAOKA's im Abstand von 15 cm bestrahlt wurden, zur Verwendung. Kulturdauer: 4 Tage bei 30°.

Rosahefesuspension:

TABELLE V.

	Kontrolle	Belichtungsdauer (Min.)		
		0	30	90
Anfangs-pH	4.0			
Pilzerntegewicht mg	139.0	105.0	91.0	90.0
	131.5	81.0	71.0	68.5
	140.5	83.5	89.0	92.0
Verhältnis	100.0	65.5	61.7	60.9

Alkoholextrakt von Rosahefezellen :(1)

TABELLE VI.

	Kontrolle	Belichtungsdauer (Min.)			Rosahefe-pulver (0.1%)
		0	30	90	
Anfangs-pH	3.8				
Pilzerntegewicht mg	131.0	98.5	102.0	106.5	75.0
	151.0	84.0	106.5	90.0	85.0
	131.0	93.0	92.0	91.0	103.0
Verhältnis	100.0	66.8	72.9	69.8	63.8

Wachstumsbeschleunigender Stoff im Filtrat der Rosahefekulturlösung :(2)

TABELLE VII.

	Kontrolle	Belichtungsdauer (Min.)				Rosahefe-kultulösung (unfiltriert)
		0	30	90	120	
Anfangs-pH	3.8					
Pilzerntegewicht mg	116.5	228.0	234.0	241.5	236.0	47.5
	112.0	241.0	223.0	236.0	242.0	49.0
	132.0	227.0	241.0	225.0	237.5	57.0
Verhältnis	100.0	193.0	193.5	194.5	198.2	42.5
Konidienbildung	−	++++	++++	++++	++++	−

Tabelle V, VI und VII zeigen, dass die von Rosahefe gebildeten, das Pilzwachstum modifizierenden Stoffe gegen ultraviolette Strahlen ganz beständig sind.

(3) Asche der Rosahefe

Versuch V

Bezüglich ihrer starken Wirksamkeit sowie der Hitzebeständigkeit erinnert uns die Wirkung der Giftsubstanz in Rosahefezellen wohl an

(1) Siehe unten.
(2) Siehe unten.

diejenige von verschiedenen Schwermetallsalzen. Es dürfte also wichtig sein zu sehen, ob die durch Glühhitze erhältliche Asche der Rosahefezellen auf das Wachstum von *Aspergillus* einwirke.

Die zu untersuchende Rosahefe wurde in gewöhnlicher Kulturlösung gezüchtet. Nach 10 Tagen wurden die Hefezellen abzentrifugiert, wiederholt mit doppeltdest. Wasser gewaschen, und dann bei 70° schnell getrocknet. 0.025 g von der so erhaltenen Trockenhefe wurden im Porzellantiegel bis zum Glühen erhitzt, und der Aschenrückstand wurde zu 25 ccm der Kulturlösung von *Aspergillus oryzae* zugesetzt. Nach 4 Tage langer Kultur wurde das Trockengewicht der Pilzernte mit demjenigen von Kontrollkultur verglichen. Das Verhältnis betrug dabei 100 : 104, woraus man wohl mit Recht schliessen kann, dass die Giftsubstanz in Rosahefezellen nichts mit dessen Aschenbestandteilen zu tun habe.

(4) Schwellenwert der Förderungs- und Hemmungswirkung

Versuch VI

In diesem Versuch habe ich den Grad der Beschleunigung bzw. der Hemmung des Wachstums von *Aspergillus oryzae* bei Zusatz verschiedener Mengen von Bierhefe- bzw. Rosahefezellen untersucht. Die zu untersuchenden Bier- und Rosahefen wurden auf gewöhnliche Kulturlösung kultiviert. Nach etwa 14-tägiger Kultur bei etwa 25° wurden die Hefezellen durch Zentrifugieren gesammelt und wiederholt mit Aqua dest. gewaschen. Die so erhaltene Hefemasse wurde durch Bestrichen auf eine Tonplatte getrocknet, und dann im Vakuumexsikkator aufbewahrt. Nachdem die Hefezellen die Feuchtigkeit gänzlich verloren hatten, wurde die Hefemasse in einem Porzellanmörser gut zerrieben. Das auf diese Weise erhaltene Hefepulver wurde in verschiedenen Mengen der Kulturlösung von *Aspergillus oryzae* zugesetzt, und zwar in folgenden Konzentrationen.

0.01%	0.005%	0.0025%	0.00125%	0.000625%	0.0003125%
(1×10^{-4}),	$(^1/_2\times10^{-4})$,	$(^1/_4\times10^{-4})$,	$(^1/_8\times10^{-4})$,	$(^1/_{16}\times10^{-4})$,	$(^1/_{32}\times10^{-4})$

Diese Kulturlösungen wurden nach 1 Stunde langer Sterilisation im Dampftopf mit Konidien von *Aspergillus oryzae* geimpft. Kulturdauer: 4 Tage bei 30°.

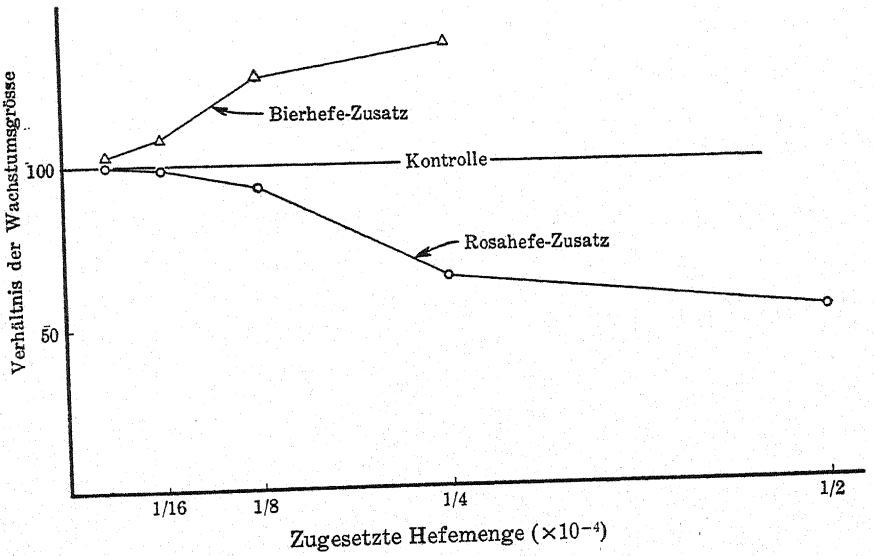


Fig. 1.

TABELLE VIII.

Zusatz von *Torula Suganii* (Rosahefe Nr. 1)

	Pilzerntegewicht[1] mg	Verhältnis	Kojisäure- menge %	Kojisäure- produktivität[2]	Titrationsacidität[3] (Anfang) 6.99 ccm.	Zuckergehalt (Anfang) 8.6 %	Ökonom. Koeff.	pH (Anfang) 3.4
Kontrolle	147.5	100.0	0.19	0.34	9.41	6.57	28.0	3.1
1 ×10⁻⁴	76.2	51.3	0.26	0.87	9.80	7.66	32.7	3.0
1/2 ×10⁻⁴	79.8	53.3	0.22	0.68	9.45	7.47	28.6	3.1
1/4 ×10⁻⁴	94.3	64.1	0.21	0.56	9.68	6.61	19.1	3.0
1/8 ×10⁻⁴	134.0	91.0	0.32	0.58	9.85	6.12	22.6	3.1
1/16×10⁻⁴	143.8	98.5	0.35	0.61	10.63	6.20	24.3	3.0
1/32×10⁻⁴	147.0	99.9	0.33	0.56	10.38	5.80	21.3	3.1

Zusatz von *Saccharomyces cerevisiae*.

					(Anfang) 7.10 ccm	(Anfang) 8.6 %		(Anfang) 3.4
Kontrolle	134.7	100.0	0.04	0.07	8.51	6.33	22.7	3.6
1/4 ×10⁻⁴	186.0	138.1	0.08	0.09	9.67	5.59	24.7	3.6
1/8 ×10⁻⁴	173.0	128.9	—	—	—	5.51	22.4	3.5
1/16×10⁻⁴	147.3	109.4	0.06	0.09	9.00	6.03	23.2	3.7
1/32×10⁻⁴	140.3	104.2	0.07	0.09	9.32	5.99	21.7	3.4

(1) Durchschnittswert von drei Parallelversuchen.
(2) Vergl. H. TAMIYA: Acta Phytochimica, **3**, 1927, 68.
(3) Titrierung mit N/10 NaOH.

Man kann aus Tabelle VIII und Fig. 1 entnehmen, dass sich die Pilzwachstumsbeschleunigung durch Bierhefepulver schon bei 0.000625%igem Zusatz erkennen lässt, während die Wachstumshemmung durch Rosahefepulver-Zusatz bei 0.000625% bzw. 0.003125% nicht so deutlich, und erst bei 0.00125%igem Zusatz eindeutig zustande kommt. Zieht man jedoch den Umstand in Betracht, dass die in solchen Zellen enthaltene wirksame Substanz nur einen kleinen Bruchteil vom ganzen Zellinhalt ausmacht, so ist es wohl zu ersehen, dass die in Frage kommende Substanz schon in äusserst kleiner Menge oder Konzentration den Wachstumseffekt hervorgebracht haben muss.

(5) Abhängigkeit der Giftwirkung von der Tiefe der Kulturlösung

Versuch VII

Wir sind weiter vor der Frage gestellt, ob der in Frage kommende Giftstoff von den abgetöteten Rosahefezellen leicht in Aussenlösung herausdiffundiere. Um diese Frage zu beantworten habe ich folgenden Versuch ausgeführt. Die Kulturlösung wurde zunächst mit den intakten Rosahefezellen beschickt, und zwar in einer Konzentration von 0.01%. Diese Lösung wurde dann in dem Kulturgefässchen nach TAMIYA[1] in verschiedener Menge (nämlich 2, 4, 6, 8 oder 10 ccm) eingefüllt, sodass die Kulturlösungen verschiedene Schichtdicke, nämlich 0.48, 0.96, 1.44, 1.92 oder 2.40 cm aufwiesen. Nach 1 Stunde langer Sterilisation im Dampftopf wurde jede Kulturlösung mit Konidien von *Asp. oryzae* geimpft, und das Erntegewicht des Pilzes wurde nach 4-tägiger Kultur bei 30° bestimmt.

Lässt man die mit Rosahefezellen beschickte Kulturlösung ruhig stehen, so sinken bald alle zugefügten Hefezellen am Boden des Kulturgefässchens ab, wie man es sowohl makroskopisch als auch mikroskopisch leicht feststellen kann.

Wie man aus Tabelle IX ersieht, wird der Grad der Wachstumshemmung bei Varierung der Tiefe der Kulturlösung gar nicht modifiziert, was uns die Annahme nahe legt, dass die Giftsubstanz in Rosahefezellen weder in oder an der Zelle festgehalten ist, sondern von derselben leicht in die Kulturlösung herausdiffundiert und das Wachstum der an Flüssigkeitsoberfläche befindlichen Pilzmyzelien affiziert.

(1) H. TAMIYA: Acta Phytochimica, **4** (1928), 229.

TABELLE IX.

Menge der Kulturlösung ccm	Kontrolle		Rosahefe-Zusatz	
	Pilzerntegewicht	Verhältnis	Pilzerntegewicht	Verhältnis
10	64.5 mg 60.0 57.5 59.0	100.0	43.5 mg 51.0 46.5 46.5	77.8
8	48.5 79.5 62.0 55.0	100.0	44.0 46.0 39.0 42.0	69.8
6	53.0 46.5 56.5 55.0	100.0	32.0 45.0 45.0 36.5	75.1
4	61.0 46.0 37.5 49.0	100.0	39.0 37.5 40.0 34.0	77.8
2	41.0 32.0 35.0 36.0	100.0	22.0 25.0 30.0 32.5	75.3

(6) Filtrierbarkeit

Versuch VIII

In diesem Versuche habe ich untersucht, ob die von Rosahefezellen ausgeschiedene Giftsubstanz auch nach Filtrierung durch einen SEITZ-Filter wirksam sei oder nicht. Eine 17-tägige flüssige Kultur von Rosahefe (siehe Versuch I, aber ohne Pepton und Agar) wurde teils nach der Verteilung der Zellen als solche in Wasser und teils nach dem gründlichen Zerreiben der Zellen im Porzellanmörser mit Meeressand (MERCK) durch den Bakterienfilter nach SEITZ filtriert, und das Filtrat bzw. der Rückstand nach der Dampfsterilisation wurden einzeln auf ihre Wirkung auf das Wachstum von *Asp. oryzae* untersucht.

TABELLE X.

Versuch mit Rosahefekulturlösung.

	Kontrolle	Filtrat-Zusatz	Rückstand-Zusatz	Zusatz von unfiltrierter Kulturlösung
Anfangs-pH	4.0			
Pilzerntegewicht mg	140.0	309.0	81.0	85.5
	139.5	309.0	85.0	84.5
	141.0	310.5	77.0	83.0
Verhältnis	100.0	220.8	57.8	60.2
Decke	Vollkomm.	Vollkomm.	Ring	Ring
Konidienbildung	—	++++	—	—

Kulturdauer: 4 Tage bei 30°.

TABELLE XI.

Versuch mit zerriebenen Zellen.

	Kontrolle	In kaltem Wasser		In heissem Wasser	
		Filtrat	Rückstand	Filtrat	Rückstand
Anfangs-pH	4.0				
Pilzerntegewicht mg	200.0	210.0	114.0	180.0	134.5
	215.0	168.0	115.5	171.0	129.5
	175.0	215.0	117.0	211.5	122.0
Verhältnis	100.0	100.5	58.7	95.3	64.5

Kulturdauer: 4 Tage bei 30°.

Es stellt sich aus Tabelle X und XI heraus, dass sich die Giftwirkung in beiden obigen Fällen nur bei Zusatz der auf Filter zurückbleibenden Rosahefemasse, gar nicht aber bei Filtratzusatz bemerken lässt, und dass durch Zugabe des Filtrates der intakten Hefesuspension sogar eine Wachstumszunahme auf das Doppelte bewirkt wird. Diese wachstumsbeschleunigende Substanz befindet sich also in der Kulturlösung und ist gut filtrierbar. Die Tatsache, dass die Beschleunigung

des Pilzwachstums beim Zusatz von wässrigem Auszug der zerriebenen Zellen kaum erkannt wird, lässt es zunächst zweifelhaft erscheinen, dass auch gewisse Zellbausteine, wie z. B. Phosphatide, wie vielfach angegeben, als wachstumsbeschleunigender Faktor in Betracht kommen. Diese Zellsubstanz scheint aber, wie der nachfolgende Versuch IX (Tabelle XII-XIII) zeigt, in phosphathaltiger Salzlösung löslich und auch filtrierbar zu sein. Übrigens hat dieselbe eine schwächere wachstumsbeschleunigende Kraft als die in Kulturlösung auftretende Substanz.

Ausserdem findet die Konidienbildung des Pilzes beim Zusatz des Filtrates von Rosahefe-Kulturlösung im Vergleich mit den anderen Fällen viel früher und reichlicher statt.

Versuch IX

Ehe man aber nach dem oben erhaltenen Ergebnis auf die Ursache der Nichtfiltrierbarkeit der in Frage kommenden Giftsubstanz schlüssig sein kann, muss man sich darüber klar machen, ob die Giftsubstanz beim Filtrieren an Gefüge des Filters adsorbiert wird oder nicht. Die Möglichkeit der Adsorption der Giftsubstanz an Filter gewinnt um so mehr an Wahrscheinlichkeit, wenn man sich daran erinnert, dass die Giftsubstanz irgend eine Schwermetallverbindung oder ein oberflächenaktiver organischer Stoff sein könne. Es geht schon aus den Arbeiten verschiedener Autoren hervor, dass die Durchlässigkeit des Filters gegen verschiedene Stoffe in hohem Masse von der H-Ionenkonzentration des Mediums abhängig ist. Es sei also von Bedeutung zu sehen, wie sich die Filtrierbarkeit der in Frage kommenden Substanz bei verschiedenen pH-Werten gestalte. Zunächst wurden folgende Pufferlösungen mit verschiedener H-Ionenkonzentration zubereitet:

10 ccm von K_2HPO_4 (M/2),	25.2 ccm von H_2SO_4 (0.1 N) . . .	pH	3.6
,,	17.2 ,, ,, . . .	pH	6.2
,,	13.64 ,, NaOH (0.1 N) . . .	pH	7.4
,,	20.18 ,, ,, . . .	pH	9.6
,,	36.36 ,, ,, . . .	pH	>9.8

Je 20 ccm von dieser Pufferlösung wurden dann mit 10 ccm Rosahefesuspension, die 0.05 g (Trockengewicht) von gut zerriebenen Rosahefezellen enthielten, versetzt, und durch den SEITZ-Filter filtriert.

Von dem Filtrat wurden 20 ccm entnommen, mit NaOH bzw. H_2SO_4 (1.0 N) neutralisiert (mit Phenolphthalein als Indikator) und dann mit doppeltdestilliertem Wasser auf 30 ccm gebracht. Diesen 30 ccm wurden dann 70 ccm von gewöhnlicher Kulturlösung zugesetzt, worin, nach dem Dampfsterilisation, die Konidien von *Asp. oryzae* ausgesät wurden.

Zugleich habe ich auch den Filterrückstand auf seinen Gehalt an der Giftsubstanz untersucht, wobei derselbe zunächst in der Pufferlösung suspendiert und, nach dem Neutralisieren und dem Verdünnen auf die ursprüngliche Konzentration, der Kulturlösung von *Asp. oryae* zugesetzt wurde. Zur Kontrolle diente die Rosahefesuspension in verschiedenen Pufferlösungen, die ohne Filtrierung gleich neutralisiert und dann, wie oben angegeben, der Kulturlösung zugesetzt wurde. Die Kulturlösung zeigten dabei folgende Zusammensetzung:

Rohrzucker	8.6 g	mit doppeltdest. Wasser auf 100 ccm gebracht.
Lösung A	15.0 ccm	
KH_2PO_4 (M/2)	15.0 „	
Filtrat od. Suspension	30.0 „	

TABELLE XII.

Filtrierung bei		Kontrolle		Zusatz von	
		ohne	mit	Filtrat	Rückstand
		Rosahefezusatz			
pH 3.6	Anfangs-pH	5.0			
	Pilzerntegewicht mg	152.5	94.5	172.0	85.0
		165.0	105.0	202.0	103.0
		162.5	99.0	209.5	105.0
	Verhältnis	100.0	62.2	121.5	61.0
pH 6.2	Anfangs-pH	5.1			
	Pilzerntegewicht mg	219.0	108.5	225.0	120.0
		196.5	85.0	218.0	112.0
		186.5	85.0	223.0	118.5
	Verhältnis	100.0	46.2	110.6	58.2

Kulturdauer: 4 Tage bei 30°.

TABELLE XII. (Fortsetzung)

Filtrierung bei		Kontrolle		Zusatz von	
		ohne	mit	Filtrat	Rückstand
		Rosahefezusatz			
pH 9.6	Anfangs-pH	3.7			
	Pilzerntegewicht mg	157.5 125.0 129.0	131.5 172.5 147.0	187.5 208.5 165.0	176.5 148.5 168.5
	Verhältnis	100.0	109.5	136.3	117.5
pH>9.8	Anfangs-pH	4.2			
	Pilzerntegewicht mg	120.5 134.5 131.5	151.5 145.0 127.0	121.0 114.0 125.0	200.0 151.0 163.5
	Verhältnis	100.0	109.5	93.2	133.1

Kulturdauer: 4 Tage bei 30°.

Zugleich habe ich die Hemmungskraft derselben Rosahefesuspension nicht in der Pufferlösung, sondern in doppeltdestiliertem Wasser geprüft:

	Kontrolle (ohne Zusatz)	Rosahefe-Zusatz
Anfangs-pH	4.0	
Pilzerntegewicht mg	164.0 195.0 161.5	64.5 99.5 85.0
Verhältnis	100.0	48.3

Kulturdauer: 4 Tage bei 30°.

TABELLE XIII.

Filtrierung bei		Kontrolle (ohne Zusatz)	Zusatz von	
			Filtrat	Rückstand
pH 6.2	Anfangs-pH	5.2		
	Pilzerntegewicht mg	101.5 102 5 99.5	148.5 153.0 147.5	71.0 71.0 68.0
	Verhältnis	100.0	147.9	69.2
pH 7.4	Anfangs-pH	5.2		
	Pilzerntegewicht mg	94.0 104.0 109.0	140.5 157.5 161.5	77.5 68.5 72.0
	Verhältnis	100.0	149.7	71.0
pH 9.6	Anfangs-pH	5.2		
	Pilzerntegewicht mg	67.5 87.5 74.0	93.5 93.5 90.5	91.0 94.0 90.0
	Verhältnis	100.0	121.2	120.1

Kulturdauer: 4 Tage bei 30°.

Aus Tabelle XII und XIII geht zunächst hervor, dass die Rosahefesuspension bei stärkerer Alkalinität (pH 9.6) schon nach kurzem Stehen ihre Giftwirkung gänzlich verliert, während die Rosahefesuspension bei kleineren pH-Werten als 7.5 ihre Giftwirkung noch ganz deutlich beibehält. Einen ganz ähnlichen Tatbestand findet man auch bei Heferückstand-Zusatz. Hierdurch hat man zu schliessen, dass die Giftsubstanz im Gegensatz zum wachstumsbeschleunigenden Stoff durch Alkalisierung zerstört wird. Bei Filtrat-Zusatz findet man in keinem Falle Hemmung, sondern nur die Beschleunigung des Pilzwachstums, woraus man wohl auf die Löslichkeit und Filtrierbarkeit der wachstumsbeschleunigenden Substanz schliessen kann. Bei alledem ist man wohl berechtigt anzunehmen, dass der wachstumshemmende Stoff bei saurer Reaktion ganz deutlich von der Filterschicht (SEITZ) adsorptiv zurückgehalten wird.

(7) Dialyse-Versuch

Versuch X

Des weiteren habe ich in diesem Versuche die Dialysierbarkeit der Giftsubstanz durch Kollodiummembran untersucht. 0.02 g von dem auf schon erwähnte Weise bereiteten Rosahefepulver wurden in 10 ccm Wasser suspendiert und in einem Kollodiumschlauch übernacht gegen fliessendes Wasser dialysiert. Die im Kollodiumschlauch stehende Hefesuspension (10 ccm) wurde dann auf gewöhnliche Weise mit 80 ccm Nährlösung und 10 ccm doppeltdest. Wasser versetzt, und nach der Sterilisation mit Konidien von *Asp. oryzae* geimpft. Zur Kontrolle diente eine Kultur mit der nicht dialysierten Rosahefenpulver-Suspension (in gleichem Mengenverhältnis). Die Kultur des Pilzes dauerte 4 Tage lang bei 30°. Wie Tabelle XIV zeigt, wies die dialysierte Rosahefenpulversuspension ganz und gar keine Giftwirkung mehr auf. Ich habe weiter ein entsprechendes Experiment mit den lebenden sowie mit den vorher bei 100° 1 Stunde lang erhitzten Rosahefen ausgeführt, und ganz dasselbe Ergebnis erzielt, wie in Tabelle XV gezeigt.

TABELLE XIV.

Rosahefe-Stammnummer		Kontrolle	Zusatz von dialysierter Suspension	Zusatz von nicht dialysierter Suspension
Nr. 1	Anfangs-pH	4.0		
	Pilzerntegewicht mg	226.0	229.0	131.0
		215.0	262.0	145.0
		213.5	258.0	115.0
	Verhältnis	100.0	111.0	59.8
Nr. 2	Pilzerntegewicht mg	193.5	269.5	113.0
		245.0	261.5	100.0
		226 5	187.0	135.0
	Verhältnis	100.0	107.9	52.3

TABELLE XV.

	Kontrolle	Zusatz von dialysierter frischer Hefe	Zusatz von dialysierter gekochter Hefe	Zusatz von nicht dialysierter frischer Hefe
Anfangs-pH	4.0			
Pilzerntegewicht mg	166.0 163.0 183.0	198.0 218.0 245.0	214.0 209.0 176.0	96.5 122.0 119.0
Verhältnis	100.0	129.1	117.0	65.9

Es ist jedenfalls merkwürdig, dass auch die lebenden Rosahefezellen nach obiger Behandlung ihre wachstumshemmende Wirkung gänzlich verloren haben.

Versuch XI

Ich habe weiter versucht zu sehen, ob die Giftsubstanz ausserhalb des Dialysierschlauchs nachweisbar sei. Zu diesem Zweck habe ich zweierlei Experimente ausgeführt. Bei einem Versuche wurde in einer Rosahefekulturlösung (in ERLENMEYER-Kolben) ein mit doppeltdest. Wasser (10 ccm) gefüllter Kollodiumschlauch eingetaucht, und nach 24 Stunden langem Stehen wurde dieses doppeltdest. Wasser wie gewöhnlich zu 80 ccm Nährlosung und 10 ccm Wasser zugesetzt, wonach die Konidien von *Asp. oryzae* beimpft wurden (Tabelle XVI). In einem anderen Versuche habe ich zunächst den Kollodiumschlauch mit der Rosahefesuspension gefüllt; dieser Kollodiumschlauch wurde dann in einer zuvor sterilisierten Kulturlösung in ERLENMEYER-Kolben von 200 ccm Inhalt eingetaucht. Nachdem der Kolben 24 Stunden lang ruhig stehen gelassen worden war, wurden darin die Konidien von *Aspergillus* eingeimpft (Tabelle XVII).

TABELLE XVI.

	Kontrolle	Zusatz vom Dialysat der Rosahefekulturlösung
Anfangs-pH	4.0	
Pilzerntegewicht mg	166.0 163.0 183.0	198.5 213.0 235.0
Verhältnis	100.0	126.2

Kulturdauer: 4 Tage bei 30°.

TABELLE XVII.

	Kontrolle	Mit dem Rosahefe enthaltenden Kollodiumschlauch	Zusatz von Rosahefesuspension
Anfangs-pH		4.0	
Pilzerntegewicht mg	897.5	998.0	491.0
	745.0	729.0	425.0
	997.0	868.5	456.0
Verhältnis	100.0	98.3	51.9

Kulturdauer: 4 Tage bei 30°.

In diesen Versuchen schlugen es uns immer fehl, das Heraustreten der Giftsubstanz aus dem Kollodiumschlauch direkt nachzuweisen, weil in beiden erwähnten Kulturen ein gleich grosses oder sogar ein besseres Pilzwachstum wie bei der entsprechenden Kontrollkultur erzielt wurde. Der Ausfall der Dialyse-Versuche muss also dadurch erklärt werden, dass die Giftsubstanz nicht durch Kollodiummembran ins Aussenmedium hinübergegangen war, sondern durch kräftige Adsorptionskraft von Kollodiummembran gänzlich zurückgehalten worden war. In Betreff der wachstumsbeschleunigenden Substanz, die in der Rosahefekulturlösung auftritt, kann man dennoch aus dem in Tabelle XVI angegebenen Ergebnis schliessen, dass gegen sie Kollodiummenmbran durchlässig ist.

(8) Löslichkeit in organischen Lösungsmitteln

Versuch XII

Es fragt sich nun, wie sich die in Betracht kommenden Stoffe gegen andere Lösungsmittel als Wasser verhalten. In diesem Versuche habe ich zunächst untersucht, ob Aceton als Lösungsmittel verwendbar ist oder nicht. Je 0.2 g von dem auf schon erwähnte Weise zubereiteten Rosahefe- bzw. Bierhefepulver wurden in 100 ccm Aceton suspendiert, und am Rückfluss auf dem Wasserbade 1 Stunde lang extrahiert. Die Hefepulversuspension wurde dann mittels gewöhnlichen Filtrierpapiers filtriert, und das Filtrat wurde auf dem Wasserbade bis zur Trockne eingedampft. Der Rückstand wurde dann

in doppeltdest. Wasser gelöst und wieder auf dem Wasserbade verdunstet. Nach abermaligem Lösen und Abdampfen wurde der Rückstand in bestimmter Menge doppeltdest. Wasser gelöst und der Kulturlösung zugesetzt.

Andererseits wurde der mit Aceton ausgezogene Hefe-Rückstand im Exsikkator mehrere Stunden lang bei 70° stehen gelassen, um das Aceton davon völlig zu verjagen. Der Rückstand wurde dann in doppeltdest. Wasser suspendiert und wie üblich zu der Kulturlösung hinzugefügt.

Nach 1 Stunde langer Sterilisation im Dampftopf wurden jeder Kulturlösung Konidien von *Asp. oryzae* beimpft, und dessen Wachstum wurde nach 4 Tage langer Kultur bei 30° ermittelt.

Aus Tabelle XVIII ersieht man ganz deutlich, dass Aceton die in Rosahefezellen enthaltene Giftsubstanz gut extrahiert, weil der Auszug aus Rosahefepulver, nicht aber derjenige aus Bierhefepulver wachstumshemmend wirkt. Der mittels Acetons extrahierte Rückstand des Rosahefepulvers erwies sich dagegen ganz wirkungslos.

TABELLE XVIII.

	Kontrolle	Extrahierung der Rosahefe mit Aceton			
		bei Zimmertemperatur		bei Siedehitze	
		Filtrat	Rückstand	Filtrat	Rückstand
Anfangs-pH	3.4				
Pilzerntegewicht mg	211.5 214.0 197.5	109.5 114.5 83.5	195.0 174.5 149.0	106.0 137.5 112.0	196.0 210.0 214.5
Verhältnis	100.0	50.1	83.2	57.0	99.6

TABELLE XIX.

	Kontrolle	Acetonextrahierung von Bierhefe		Zusatz von frischer Bierhefe
		Filtrat	Rückstand	
Anfang-pH	4.0			
Pilzerntegewicht mg	104.5 115.0 104.0 109.0	111.0 105.0 111.0 110.0	258.5 240.5 276.0 255.0	167.0 160.0 167.0 177.0
Verhältnis	100.0	101.0	238.1	155.2

Die Tatsache, dass das sogenannte Bios, das in Bierhefezellen enthalten ist, in Aceton unlöslich ist, wird hierbei dadurch bewiesen, dass der Auszug aus Bierhefepulver keine wachstumsbeschleunigende Wirkung aufweist, während der Rückstand noch eine starke Wirksamkeit beibehält (Tabelle XIX). Dass der Rückstand des Rosahefepulvers hierbei keine Bios-Wirkung zeigte, könnte darauf beruhen, dass er wegen der unvollkommenen Acetonextraktion noch nicht völlig vom Hemmungsstoff befreit war.

Versuch XIII

In diesem Versuche habe ich andere organische Lösungsmittel, nämlich Äthylalkohol, Petroläther, Äther, Benzol, CS_2 und Chloroform, auf ihre Extraktionsfähigkeit gegen die in Rosahefezellen enthaltene Giftsubstanz untersucht. Das angewandte Verfahren war ganz wie vorher, nur die Extraktionsdauer war stets 4 Stunde. Die erhaltenen Resultate sind in Tabelle XX zusammenfassend angegeben.

Aus Tabelle XX ist zu entnehmen, dass unter den untersuchten Lösungsmitteln nur Äthylalkohol und Chloroform gegen Giftstoff sicher extraktionsfähig sind, weil dadurch der Heferückstand ganz unschädlich gemacht wird, und zugleich der Auszug sehr deutlich wachstumshemmend wirkt. Hingegen tritt bei der Extraktion mittels Äthylalkohols die wachstumsbeschleunigende Wirkung des Rückstandes deutlich hervor, was im Zusammenhang mit dem Befunde BOKORNYS,[1] dass das Bios nicht alkohollöslich ist, sehr bemerkenswert ist.

Es bleibt hier allerdings dahingestellt, ob andere Lösungsmittel die Giftsubstanz wirklich extrahieren oder nicht, weil es immer die Möglichkeit gibt, dass solche Chemikalien trotz wiederholtem Vertreibungsversuch hartnäckig im Rosahefepulver zurückbleiben, um dort noch mehr oder minder auf das Pilzwachstum giftig zu wirken.

Versuch XIV

Es soll hier festgestellt werden, ob die Giftwirkung der Rückstände wirklich vom Zurückbleiben der Giftsubstanz oder sonst von der Spur des Lösungsmittels, die von Rückständen festgehalten werden könnte, bedingt sei. Die Rosahefezellen wurden zunächst auf schon erwähnte Weise mit Benzol, Äther, Petroläther oder CS_2 vorbehandelt, und dann der Rückstand wiederum mit Aceton 4 Stunden lang extra-

(1) Th. BOKORNY: Centralb. f. Bakt., Abt., II. **19**, 1907, 331.

TARELLE XX.

Lösungsmittel		Kontrolle	Filtrat durch		Hefe-Rückstand
			Filtrierpapier	SEITZ-Filter	
	Anfangs-pH	4.0			
Äthyl-alkohol	Pilzernte-gewicht mg	109.0	94.5	82.0	205.0
		124.5	74.0	88.0	191.0
		123.0	76.0	102.0	201.5
	Verhältnis	100.0	68.6	77.8	167.6
Chloro-form	Pilzernte-gewicht mg	178.5	95.0	125.0	258.5
		173.0	52.0	99.0	175.0
		186.5	119.0	129.0	228.0
	Verhältnis	100.0	62.9	65.6	104.3
Petrol-äther	Pilzernte-gewicht mg	177.5	130.0	130.5	140.5
		189.0	100.5	127.0	117.5
		156.0	108.0	122.5	118.0
	Verhältnis	100.0	64.8	72.7	71.9
Äther	Pilzernte-gewicht mg	169.5	95.0	130.0	136.5
		159.5	73.0	122.0	101.0
		145.0	68.0	108.0	98.0
	Verhältnis	100.0	49.9	78.2	70.8
Benzol	Pilzernte-gewicht mg	146.0	102.5	107.0	80.5
		147.5	102.5	107.5	129.0
		157.0	114.5	70.0	99.0
	Verhältnis	100.0	79.2	63.1	68.5
CS_2	Pilzernte-gewicht mg	173.0	67.0	89.0	75.0
		198.5	50.0	107.0	87.0
		178.5	45.0	97.0	91.0
	Verhältnis	100.0	29.5	51.5	45.0

hiert, und wie vorher wurden die Auszüge und die Rückstände einzeln auf ihre Wirkung auf *Aspergillus*-Wachstum geprüft.

Wie man aus Tabelle XXI ersieht, haben die Rückstände, welche nach Vorbehandlung mit CS_2, Benzol, Äther, oder Petroläther immer noch starke Giftwirkung aufwiesen, durch nochmalige Extraktion mit

TABELLE XXI.

Vorbehandelt mit		Kontrolle	Acetonextraktion von Mutterrückstand		Mutterrückstandand
			Filtrat	Rückstand	
	Anfangs-pH	4.0			
CS_2	Pilzerntegewicht mg	174.5 160.5 188.0	115.0 120.5 140.5	190.5 186.0 215.0	59.5 67.0 51.5
	Verhältnis	100.0	71.9	113.1	34.0
Äther	Pilzerntegewicht mg		119.0 109.0 91.5	193.5 223.0 180.0	113.0 118.0 154.0
	Verhältnis		61.2	114.0	73.6
	Anfangs-pH	4.0			
Benzol	Pilzerntegewicht mg	235.0 185.0 243.0	116.0 119.0 149.0	243.5 351.0 281.0	162.0 135.0 152.0
	Verhältnis	100.0	57.0	132.1	67.7
Petroläther	Pilzerntegewicht mg		158.0 188.0 188.0	260.0 180.5 238.0	185.0 151.0 135.0
	Verhältnis		80.6	102.0	71.3

Kulturdauer: 4 Tage bei 30° C.

Aceton ihre Giftigkeit gänzlich verloren, während andererseits der Aceton-Auszug derselben stets noch eine nicht unbeträchtliche Giftwirkung zeigt. Es lässt sich also zeigen, dass die mit den untersuchten Lösungsmitteln vorbehandelten Rückstände noch reichliche Menge Giftsubstanz enthalten.

Aus einer neueren Arbeit von NIELSEN[1] geht es hervor, dass das Rhizopin, ein von gewissen *Rhizopus*-Arten gebildeter und zwar auf das Wachstum von *Avena*-Koleoptile stark befördernd wirkender Stoff, bei der Extraktion mit gebräuchlichem Äther, nicht aber bei Anwendung des mit Ferrosulfat und CaO sorgfältig gereinigten Äthers, irreversibel zerstört wird. Dass unsere Giftsubstanz auch bei Anwendung des nach NIELSEN gereinigten Äthers, und zwar sowohl heiss wie auch kalt, gut extrahiert wird, ersieht man aus nachstehender Tabelle.

(1) N. NIELSEN: Jahrb. f. wiss. Bot., **73**, 1930, 125.

TABELLE XXII.

	Kontrolle	Extrahierung bei				Rosahefe-Zusatz
		Zimmertemperatur		Siedepunkt (3 Std.)		
		Filtrat	Rückstand	Filtrat	Rückstand	
Anfangs-pH	3.8					
Pilzerntegewicht mg	258.0 170.5 266.5 224.5	114.0 102.0 148.5 121.5	225.0 241.0 226.5 245.0	72.0 94.5 66.5 81.5	242.5 215.0 276.5 240.0	96.5 97.5 89.5 119.5
Verhältnis	100.0	52.8	100.9	34.2	105.9	43.8

Kulturdauer: 4 Tage bei 30°.

Versuch XV

Eine etwa eine Monate lang bei 25° gezüchtete flüssige Kultur der Rosahefe wurde zunächst durch Zentrifugierung von Zellen befreit. Man schüttelte dann das klare Zentrifugat mit Äther in einem Scheidetrichter, wobei eine schleimige Schicht zwischen Äther und Wasser entstand. Die drei Flüssigkeitsschichten wurden sorgfältig getrennt abgefangen, und nachdem davon der Äther vollständig vertrieben worden ist, wurde jede Fraktion mit destilliertem Wasser auf das Anfangsvolumen gebracht. Je 20 ccm von diesen Lösungen wurden wie üblich zu 80 ccm von gewöhnlicher Kulturlösung zugesetzt und dann auf übliche Weise geschah Sterilisation und Aussaat der Konidien. Kulturdauer: 4 Tage bei 30°.

TABELLE XXIII.

	Kontrolle	Äther-Fraktion	Schleimige Fraktion	Zentrifugat-Fraktion		Zentrifugat ohne Ätherbehandlung
	Dest. Wasser-Zusatz			ohne Filtrierung	SETZ-Filtrat	
Anfangs-pH	4.0					
Pilzerntegewicht mg	165.0 181.5 185.0	57.5 57.5 63.0	115.0 98.0 101.5	182.0 187.0 187.5	239.0 251.5 267.0	106.0 90.0 89.0
Verhältnis	100.0	33.5	59.2	104.5	142.0	53.4
Konidienbildung	—	—	—	—	++++	—

Angesichts der Resultate dieses Versuchs (Tabelle XXIII) liegt es nahe anzunehmen, dass die in Frage kommende ätherlösliche Giftsubstanz reichlich gelöst in der Kulturlösung vorhanden ist, obwohl man die gänzliche Abwesenheit der Hefezellen im untersuchten Zentrifugat vorläufig nicht behaupten kann. Überzeugend wurde es aber hier dargetan, dass der neben der Giftsustanz auftretende, auf Wachstum und Sporenbildung des Pilzes fördernd wirkende Stoff nicht ätherlöslich ist.

(9) Adsorption

Versuch XVI

Um weiteren Aufschluss über die chemische Natur des Hemmungsstoffes zu gewinnen, habe ich einige Adsorptionsversuche angestellt, wobei als Adsorbens Kaolin und Pilzmyzelien von *Asp. oryzae* gebraucht wurden.

(a) *Kaolin*

Zu 80 ccm von einer 1 Monate alten Kultur der Rosahefe wurden 1.5 g Kaolin zugesetzt und einige Stunden lang auf Maschine geschüttelt. Durch Zentrifugierung wurde dann die Kulturlösung vom Kaolin abgetrennt, wobei eine etwas getrübte, vielleicht nicht völlig zellenfreie Flüssigkeit erhalten wurde. Zum Vergleichszweck wurde auch eine sterile Nährlösung auf dieselbe Weise mit Kaolin behandelt. Je 20 ccm von dieser beiden Lösungen wurden mit der Kulturlösung auf 100 ccm gebracht und dann mit den Konidien von *Asp. oryzae* beimpft. Kultur dauerte 4 Tage lang bei 30°.

TABELLE XXIV.

	Kontrolle	Durch Zentrifugierung von Rosahefezellen abgetrennte Kulturlösung		Rosahefekultur (ohne Zentrifugierung)		SEITZ-Filtrat der Rosahefekultur
		Kaolin-Behandlung	Ohne Behandlung	Kaolin-Behandl.	Ohne Behandl.	
Anfangs-pH			3.8			
Pilzerntegewicht mg	182.5 135.0 175.0 182.0	176.0 219.5 190.5 161.5	40.5 50.5 46.0 45.0	186.5 192.0 177.5 163.5	57.0 57.5 60.0 52.0	303.5 311.0 312.0 307.5
Verhältnis	100.0	110.9	27.0	106.7	32.1	182.9

Wie aus Tabelle XXIV ersichtlich, wurde die Giftsubstanz durch Kaolinbehandlung gänzlich von der Kulturlösung beseitigt. Die bei diesem Versuch erhaltenen Kaolinrückstände wurden weiter zu den Kulturlösungen zugesetzt und auf ihre Wirkung auf Pilzwachstum geprüft. Wie in Tabelle XXV gezeigt, war hierbei keine Hemmung, sondern merkwürdigerweise immer gewisse Beschleunigung des Pilzwachstums konstatiert. Gestützt auf solche Tatbestände könnte man jedenfalls schliessen, dass das Kaolin gegen die in Frage kommende Giftsubstanz stark adsorbierend wirkt.

TABELLE XXV.

	Kontrolle (Zusatz von unbehandeltem Kaolin)	Durch Zentrifugierung von Rosahefezellen abgetrennte Kulturlösung (Kaolin-Zusatz)	Rosahefe-kultur (Kaolin-Zusatz)
Anfangs-pH	3.8		
Pilzernte-gewicht mg	165.0 197.5 155.0	223.0 252.0 230.5	173.5 221.5 211.5
Verhältnis	100.0	136.3	117.2

Was nun die Adsorbierbarkeit der neben der Giftsubstanz vorkommenden auf Pilzwachstum beschleunigend wirkenden Substanz anlangt, so habe ich mit der durch SEITZ-Filter abfiltrierten Kulturlösung, in welcher, wie schon gezeigt, keine Giftsubstanz mehr enthalten war, in oben angegebener Weise experimentiert. (Siehe Tabelle XXVI.)

TABELLE XXVI.

	Kontrolle	Rosahefe-kulturlösung	Filtrat durch SEITZ-Filter	
			Behandlung mit Kaolin	Ohne Behandlung
Anfangs-pH	3.8			
Pilzernte-gewicht mg	150.5 129.0 136.0 157.5	53.0 53.0 60.5 57.0	276.0 277.5 287.0 303.5	253.5 270.5 273.0 260.5
Verhältnis	100.0	39.1	200.0	184.9

Kulturdauer: 4 Tage bei 30°.

Das erhaltene Resultat zeigt uns nun eindeutig, dass die wachstumsbeschleunigende Substanz von Kaolin so gut wie gar nicht adsorbiert wird.

(b) *Pilzmyzelien*

Die gewöhnliche Kultur von *Asp. oryzae* in PETRIschale wurde 30 Stunden nach dem Aussaat der Konidien abzentrifugiert. Die erhaltenen jüngen Myzelien von *Asp. oryzae* (0.115 g in Trockengewicht) wurden nach wiederholtem Waschen mit dest. Wasser mit 10 ccm dest. Wasser in 40 ccm Rosahefekulturlösung getan und nach einige Stunden langer Schüttelung übernacht stehen gelassen. Die Lösung wurde dann abgeschleudert und zugleich mit dem Bodensatz (Myzelien und Rosahefezellen) auf ihre Wirkung auf Pilzwachstum geprüft. Zur Kontrolle diente die Pilzkultur mit dem Zusatz von der ebenfalls mit Myzelien behandelten sterilen Nährlösung. Kulturdauer: 4 Tage bei 30°.

TABELLE XXVII.

	Zusatz von steriler Nährlösung		Rosahefekultur behandelt mit Myzelien		Rosahefekultur ohne Behandlung mit Myzelien
	Zentrifugat	Myzelien	Zentrifugat	Myzelien samt Rosahefezellen	
Anfangs-pH	3.8				
Pilzerntegewicht mg	191.0	226.0	150.0	176.5	52.0
	175.0	185.0	157.5	172.0	49.0
	196.0	219.0	167.5	210.5	46.0
Verhältnis	100.0	112.1	84.5	99.4	26.1

Aus Tabelle XXVII ist ersichtlich, dass die Pilzmyzelien, wenn auch in viel geringerem Umfang als das Kaolin, die in Betracht kommende Giftsubstanz adsorbieren, weil die Hemmung des Pilzwachstums bei Zusatz vom mit Myzelien vorbehandelten Zentrifugat nur 15% beträgt, während ohne diese Vorbehandlung 74%ige Wachstumshemmung stattfindet. In Betracht zu ziehen ist hierbei der Umstand, dass die Menge der als Adsorbens verwendeten Pilzmyzelien (im Gewicht) nur ein Zehntel der von Kaolin ausmacht.

(10) Eluierung der an Kaolin adsorbierten Giftsubstanz

Versuch XVII

Zum weiteren Nachweis dafür, dass die Giftsubstanz durch Kaolin adsorbiert wird, erschien es uns wichtig, die Eluierung der adsorbierten Substanz zu versuchen. Der Umstand, dass der in Frage kommende Stoff, wie schon angegeben, durch Alkalisierung des Milieus inaktiviert wird, macht den bei Kaolinadsorption üblichen Eluierungsversuch mit alkalischer Lösung nicht anwendbar. Als Extraktionsmittel kam also der Äther zur Anwendung, der sich sofern aus meinem Versuch hervorgeht, als sehr wirksames Lösungsmittel für die Giftsubstanz zeigt.

Das die Giftsubstanz adsorbierte Kaolin wurde zunächst mit dest. Wasser gut gewaschen, und bei 70° getrocknet. Die Kaolinmasse wurde dann fein pulverisiert und mit Äther ausgezogen. Der Äther wurde sofort oder nach 12-stündigem Stehen durch Filtrierpapier[1] filtriert und abgedampft, und der Rückstand in dest. Wasser von demselben Volumen wie die anfangs gebrauchte Rosahefekultur gelöst. 20 ccm von dieser Lösung wurden zu 80 ccm Kulturlösung zugesetzt, worin dann, nach der Sterilisation, Konidien von *Asp. oryzae* eingeimpft wurden. Als Kontrolle wurden auch Ansätze mit dem Ätherauszug des reinen Kaolins angestellt.

TABELLE XXVIII.

	Kontrolle	Ätherauszug aus behandeltem Kaolin		Kulturlösung der Rosahefe
		Sofort	12 Std. Extraktion	
Anfangs-pH	4.0			
Pilzerntegewicht mg	148.0	88.5	67.5	82.5
	121.5	84.0	65.5	77.0
	129.5	87.0	67.0	75.0
Verhältnis	100.0	65.0	50.0	58.8

Wie schon erwähnt, wird die Giftsubstanz in wässriger Lösung bei der Filtration durch SEITZ-Filter gänzlich von demselben zurück-

(1) Die Giftsubstanz in Ätherlösung geht das Filtrierpapier leicht durch.

gehalten, sodass das Filtrat nicht mehr wirksam wird. Ein SEITZ-Filter wurde zunächst mit Äther wiederholt gewaschen. Man filtrierte dann 40 ccm reinen Äther und legte es als Kontrollprobe beiseite. Durch denselben Filter wurden dann 20 ccm Rosahefekultur filtriert, wobei die ganze Menge der Giftsubstanz an Filter adsorbiert wurde. Man liess dann 40 ccm des Äthers wiederholt durch den Filter fliessen, und von diesem Ätherauszug sowie auch von der Kontrollprobe wurde der Äther vollständig abgedampft. Die von beiden Ansätzen erhaltenen Rückstände wurden in je 20 ccm dest. Wasser mit 80 ccm Kulturlösung gelöst. Der Vergleich dieser zwei Portionen ergab folgende Resultate.

TABELLE XXIX.

	Kontrolle	Ätherextrakt des Filters	Kulturlösung der Rosahefe
Anfangs-pH	4.0		
Pilzerntegewicht mg	211.0	122.5	94.0
	227.0	135.5	102.0
	225.5	111.5	97.5
Verhältnis	100.0	55.7	44.2

Kulturdauer: 4 Tage bei 30°.

Aus Tabelle XXVIII und XXIX geht mit Deutlichkeit hervor, dass die an Kaolin oder SEITZ-Filter adsorbierte Giftsubstanz mit Äther eluiert wird.

Physiologische Verhalten der Giftsubstanz

(1) Wirkung der Giftsubstanz auf Schimmelpilzmyzelien von verschiedenem Alter

Versuch XVIII

Es geht aus Untersuchungen mehrerer Autoren hervor, dass die Empfindlichkeit der Schimmelpilzmyzelien gegen verschiedene Gift- oder Reizstoffe jenach dem Alter sehr verschieden ist. Im allgemeinen sind jüngere Hyphen viel empfindlicher als die älteren, sodass

die Substanzen, die auf jüngere Hyphen giftig einwirken, sich für erwachsene Myzelien oft wirkungslos oder vielmehr günstig zeigen. Es besteht also die Möglichkeit, dass sich der von Rosahefezellen gebildeten Giftstoff gegen Schimmelpilze jenach dem Alter der Kultur recht verschieden verhält.

Zur Erprobung gelangten hier 2- und 4-tägige Deckenkultur von *Asp. oryzae*. Die erhaltene Resultate sind in Tabelle XXX zusammengestellt.

TABELLE XXX.

Versuch mit 4-tägigen Pilzdecken.

	Kontrolle	Zusatz von abgetöteten Rosahefezellen (0.01%)
Pilzerntegewicht g	2.048	1.651
	1.428	1.593
	1.517	1.545
	1.365	1.249
Zuwachsverhältnis	100.0	93

(Anfangsgewicht der Pilzdecke: durchschnittlich 0.437 g.)

TABELLE XXXI.

Versuch mit 2-tägigen Pilzdecken.

	Vor Übertragung 24 Stunden auf dest. Wasser gelegt		Unmittelbare Übertragung (Ohne Waschung)	
	Kontrolle	Rosahefezusatz	Kontrolle	Rosahefezusatz
Pilzerntegewicht mg	116.5	114.5	106.5	104.0
	124.0	113.5	112.0	114.0
	131.5	125.0	119.5	96.5
	122.5	118.0	105.5	116.5
	113.0	126.5	116.0	106.5
	119.5	121.0	98.0	95.0
	111.0	110.0	101.0	101.0
	120.5	107.0	107.0	110.0
Zuwachsverhältnis	100.0	96.8	100.0	96.4

(Anfangsgewicht der Pilzdecke: durchschnittlich 30.8 mg.)

(1) Die Kulturmethode war nach TAMIYA, wobei die fertige Decke, ehe sie zum Versuch genommen wurde, übernacht auf destilliertem Aqua dest. gelegt wurde. Vergl. H. TAMIYA: Acta Phytochimica, **4**, 1928, 81.

Merkwürdigerweise stellt es sich hierbei heraus, dass die Giftwirkung der Rosahefezellen auf fertige Decken viel undeutlicher ist als es bei Sporenkultur immer der Fall ist.

Versuch XIX

Je 10 ccm von einer dichten (10000 pro 1 ccm) Suspension der *Aspergillus*sporen in gewöhnlicher Kulturlösung wurden teils mit 2.5 ccm sterilem Wasser, teils mit 2.5 ccm vorher erhitzter Rosahefesuspension (0.01 %) versetzt, und bei 30° stehen gelassen. Nach verschiedenen Zeitintervallen wurde der Prozentsatz der ausgekeimten Konidiosporen und die durchschnittliche Länge der erwachsenen Hyphen gemessen. Die Resultate sind in folgender Tabelle zusammengestellt.

TABELLE XXXII.

Zeit in Std.	Kontrolle		Rosahefezusatz	
	Hyphenlänge μ	Keimungs-prozent	Hyphenlänge μ	Keimungs-prozent
9	47.87	78.1	56.85	72.6
11	75.07	79.3	80.78	80.6
13	128.66	86.2	98.19	86.5
15	174.08	100.0	132.19	100.0
17	lang gewachsen		lang gewachsen	

Bezüglich des Prozentsatzes der Keimung bis 15 Stunden nach der Impfung, wo schon alle Konidien ausgekeimt hatten, war gar kein Unterschied zwischen beiden Kulturreihen zu bemerken. Was aber die durchschnittliche Länge der Pilzhyphen betrifft, so ist ein gewisser Unterschied zwischen beiden Kulturen zu finden. In früheren Kulturstadien waren die Hyphen bei Rosahefezellen-Zusatz merkwürdigerweise etwas länger als bei Kontrollkultur, während in späteren Kulturstadien die Hyphen in der mit Rosahefe versetzten Kultur immer langsamer wuchsen, bis sie schliesslich (15 Stunden nach der Impfung) ein bedeutend schlechteres Wachstum als die Kontrollprobe zeigten. Diese Sachlage ist auch in Fig. 2 veranschaulicht.

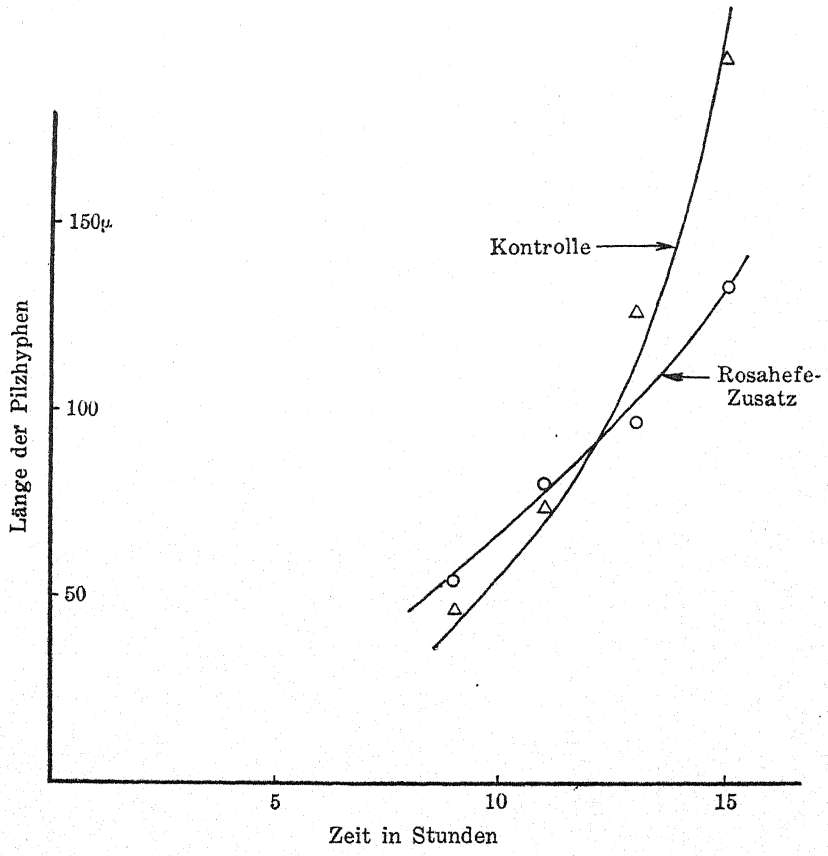


Fig. 2.

Versuch XX

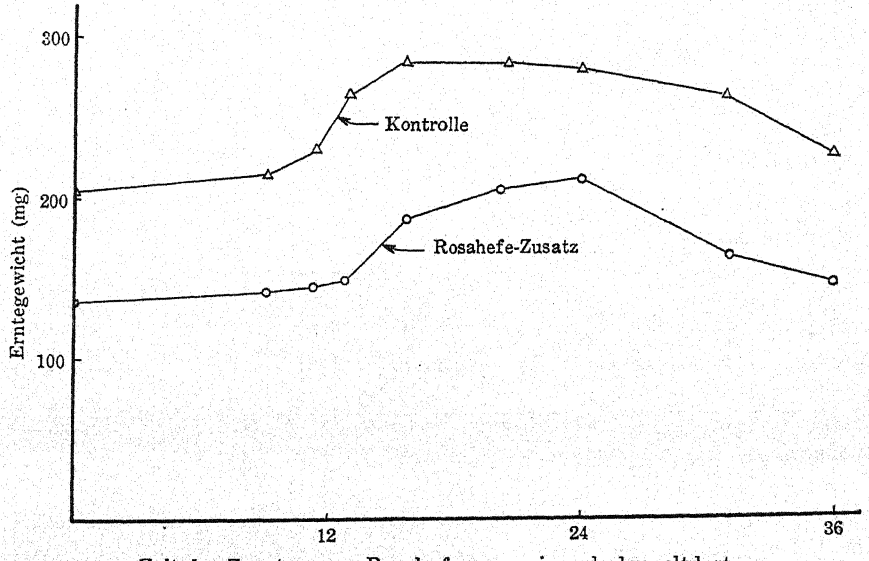
Der obige Versuch lehrt uns nun, dass die von Rosahefezellen gebildete Substanz nicht auf Konidienkeimung, sondern auf das Wachstum der ganz jüngen Pilzhyphen hemmend wirkt. Um ferner die Beziehung zwischen dem Alter der Hyphen und der Empfindlichkeit gegen die von Rosahefe gebildete Giftsubstanz genauer kennen zu lernen, habe ich folgende Experimente ausgeführt.

Je 20 ccm Kulturlösung für *Aspergillus* wurden in ERLENMEYER-Kolben (50 ccm Inhalt) getan, und nach der Sterilisation wurde die Konidiensuspension beimpft. (Kulturtemperatur: 30°.) Nach bestimmten Zeitintervallen wurden zu jeden Kulturansätzen je 5 ccm

sterilisierte Rosahefesuspension oder sterilisiertes dest. Wasser mittels steriler Pipette zugesetzt. Nachdem man die Kolben je 5 Minuten lang mit Hilfe einer Schüttelmaschine geschüttelt hat, wurden die Kulturen wieder im Thermostat stehen gelassen, und am 4. Tage nach der Impfung wurde die Pilzernte ermittelt. Die Resultate sind in Tabelle XXXIII und Fig. 3 dargestellt.

TABELLE XXXIII.

Zeit des Zusatzes nach der Impfung (Std.)	0	9	11	13	16	20	24	31	36
Erntegew. (mg) bei Kontrolle	230.5	233.5	205.5	279.5	330.5	325.0	329.0	311.5	188.0
	185.0	213.0	226.0	241.5	270.0	246.0	281.5	253.5	205.5
	198.5	218.0	273.5	284.5	272.0	296.0	247.0	240.0	203.0
Verhältnis	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Erntegew. (mg) bei Zusatz der Giftsubstanz	124.0	146.0	148.0	162.5	170.0	204.0	208.0	156.0	186.0
	140.0	117.0	156.0	117.0	203.5	214.0	206.0	174.5	145.6
	141.0	168.0	132.0	188.0	200.0	209.0	239.5	175.0	120.5
Verhältnis	65.9	64.9	61.8	58.0	65.7	72.3	76.2	62.8	75.8



Zeit des Zusatzes von Rosahefesuspension od. doppeltdest. Wasser nach der Impfung (Std.)

Fig. 3.

Bei den Kulturansätzen, zu welchen 9-17 Stunden nach der Impfung Rosahefe zugesetzt wurde, zeigten die Pilzdecken ganz wie bei früheren Versuchen nur ringförmiges Anwachsen, sonst wurden vollkommene Decken gebildet, die aber viel dünner waren als die der Kontrolle. Die Gegenüberstellung der erhaltenen Resultate mit denen des vorherigen Versuchs zeigt, dass die Giftsubstanz besonders giftig für junge Pilzmyzelien ist. Beachtenswert ist übrigens die Tatsache, dass das Wachstum des Pilzes durch Schüttelung nach der Keimung nicht gehemmt, sondern eher um etwa $^1/_2$-fachen Betrag beschleunigt wird.

Versuch XXI

In diesem Versuch wurde ein ähnliches Experiment wie oben mit der durch organische Lösungsmittel (Äthylalkohol und Chloroform) extrahierten Substanz ausgeführt. Die Extrahierung und Fraktionierung der Giftsubstanz geschah ganz auf die schon geschilderte Weise. Nach 16 Stunden langem Verweilen nach der Konidienimpfung wurde die wässrige Lösung des zu prüfenden Materials in *Aspergillus*kultur getan und nach 4 Tagen wurde der Pilzertrag bestimmt. Wie Tabelle XXXIV zeigt, ergab dieser Versuch ein ganz demjenigen des vorherigen Experimentes analoges Resultat.

TABELLE XXXIV.

Zeit d. Zusatzes nach d. Impfung Std.	0		16				
Probe	Kontrolle	Zusatz der Rosahefe	Kontrolle	Äthylalkohol-Auszug		Chloroform-Auszug	
				Filtrat	Rückstand	Filtrat	Rückstand
Anfangs-pH	4.0						
Erntegewicht mg	196.0	73.0	192.0	60.5	222.5	96.0	212.0
	220.0	61.0	189.0	59.5	185.5	90.5	152.0
	199.5	62.5	178.5	50.5	180.5	80.5	152.0
Verhältnis	100.0	31.9	100.0	30.5	105.2	47.7	92.2

Kulturdauer: 4 Tage bei 30°.

TABELLE XXXV.

Kulturdauer (Tage)	Zusatz	Erntegew.	Verhältn.	Kojisäure %	Kojisäure-Produktiv.	Titrations-Acidität[1] (Anfang) 8.69 cc.	pH (Anfang) 4.0
3	Ohne Zusatz	50.0 mg	100.0	~	~	8.85	4.0
		50.0		~	~	8.79	3.7
		72.0		0.01	0.03	8.55	3.6
	Rosahefe-Zusatz	33.0	40.9	~	0	8.69	3.6
		18.5		~	0	8.79	3.6
		19.0		~	0	8.75	3.6
5	Ohne Zusatz	186.0	100.0	0.35	0.47	11.30	4.0
		255.0		0.78	0.76	13.47	4.0
		199.0		0.30	0.38	10.72	4.0
	Rosahefe-Zusatz	91.0	50.2	0.21	0.58	9.99	3.9
		135.0		0.66	1.22	12.05	3.9
		108.0		0.25	0.58	10.19	4.0
8	Ohne Zusatz	256.0	100.0	2.80	2.73	21.75	4.3
		248.5		3.50	3.53	21.57	4.3
		237.0		3.20	3.37	20.48	4.1
	Rosahefe-Zusatz	121.0	62.9	2.80	5.79	19.20	4.3
		208.5		4.00	4.80	22.57	4.3
		137.5		3.50	6.38	18.18	4.2
12	Ohne Zusatz	273.0	100.0	1.50	1.37	17.13	4.1
		235.0		1.00	1.06	13.65	4.2
		289.0		2.25	1.94	19.07	4.2
	Rosahefe-Zusatz	336.5	115.6	0.50	0.37	11.23	4.2
		330.0		0.70	0.57	12.29	4.2
		257.5		2.13	2.07	18.18	4.3
20	Ohne Zusatz	200.5	100.0	0	0	10.47	4.2
		221.5		0	0	10.52	4.3
		202.5		0	0	10.82	4.3
	Rosahefe-Zusatz	232.0	104.7	0	0	9.76	4.4
		215.0		0	0	9.97	4.3
		207.0		0	0	10.14	4.5

(1) Titrierung mit N/10 NaOH.

Versuch XXII

Angesichts der Tatsache, dass die in Frage kommende Giftsubstanz nur auf die jungen Myzelien, nicht aber auf erwachsene Pilzdecken wachstumshemmend wirkt, haben wir uns noch die Frage zu beantworten, ob und in wieweit der Zusatz dieses Stoffes auf die Dauer auf den Stoffwechsel von *Asp. oryzae* einflussgebend sei. Der Vergleich der mit Rosahefe (0.01 %) versetzten Kultur mit der Kontrollkultur bei längerer Kulturdauer ergab nun die Resultate, die in der Tabelle XXXV und Fig. 4 dargestellt sind.

Der Wachstumsunterschied zwischen beiden Kulturreihen verschwand schon nach 12 Tagen, wobei das Erntegewicht der mit Rosahefe versetzten Kulturen sogar etwas grösser ausfiel als das der Kontrollkultur. Dieser Tatbestand ist wohl so zu deuten, dass einerseits die Kulturlösung der Kontrollkultur wegen ihres üppigeren Wachstums schneller in Zustand von sog. „Staling" (Mangel der Nährstoffe, Anhäufung der schädlichen Stoffwechselprodukte u.s.w.) geriet, und andererseits dass die Pilzmyzelien mit dem Wachstum gegenüber der Giftsubstanz der Rosahefe resistenter wurden, indem zugleich die wachstumsbeschleunigende Wirkung von derselben immer mehr in den Vordergrund trat.

Solche Erscheinung tritt besonders ausgeprägt beim Kulturversuch von *Rhizopus nigricans* ein, dessen Wachstum zwar durch die biosartige Substanz in Rosahefe viel deutlicher beschleunigt zu werden scheint als das von *Aspergillus*.

So lieferte z.B. die mit Rosahefe versetzte Kultur von *Rhizopus* schon am 6. Tage nach der Impfung grössere Pilzernte als die Kontrollkultur. (Vergl. Tabelle XXXVI.)

Es ergibt sich noch aus dem Experiment mit *Asp. oryzae*, dass die Kojisäureproduktion von diesem Pilz durch Zusatz der Giftsubstanz etwas vergrössert wird. Den Maximumwert der Kojisäureproduktivität fand man bei 8 tägiger Kultur, während bei 20 tägiger Kultur die Säure durch Wiederverarbeitung seitens des Pilzes nicht mehr nachweisbar wurde.

(2) Abhängigkeit der Wirkung von der Natur der N-Quelle

Versuch XXIII

Um zu sehen, ob und wie die Empfindlichkeit des Pilzes gegen die von Rosahefe gebildete Giftsubstanz von dem N-Stoffwechsel des

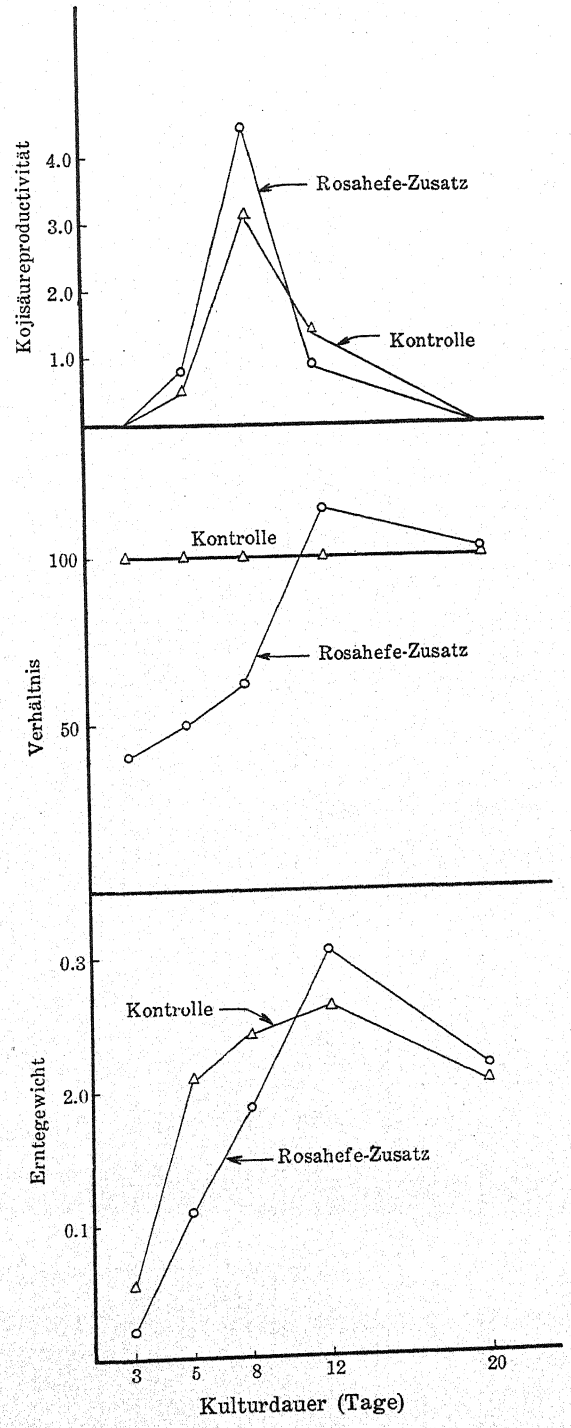

Kojisäureproductivität
4.0
3.0
2.0
1.0
Rosahefe-Zusatz
Kontrolle
Verhältnis
100
50
Kontrolle
Rosahefe-Zusatz
Erntegewicht
0.3
2.0
0.1
Kontrolle
Rosahefe-Zusatz
3
5
8
12
20
Kulturdauer (Tage)

Fig. 4.

TABELLE XXXVI.

Rhizopus nigricans

Kulturdauer (Tage)		Kontrolle	Rosahefezusatz (0.05%)
2	Anfangs-pH		4.0
	Pilzerntegewicht mg	40.5 40.5 37.0	19.0 23.5 20.5
	Verhältnis	100.0	53.4
9	Pilzerntegewicht mg	72.0 74.0 70.0	97.5 95.0 89.0
	Verhältnis	100.0	130.0

Kulturtemperatur: 25°.

betreffenden Pilzes abhängig sei, habe ich in diesem Versuch statt des Ammoniumnitrates in bisheriger Kulturlösung KNO_3 bzw. $(NH_4)_2HPO_4$ als N-Quelle gegeben, und zwar unter folgender Dosierung:

(Die absolute N-Menge in diesen beiden Kulturreihen beträgt also die Hälfte von der bisherigen).

Rohrzucker	8.6	g
KH_2PO_4	0.17	,,
K_2HPO_4	0.218	,,
$MgSO_4$	0.2	,,
$(NH_4)_2HPO_4$	0.661	,,
oder KNO_3	1.011	,,
Rosahefesuspension in verschiedener Konzentr.	20 cc	
$FeCl_3$ (0.5%)	1 Tropf	

mit doppeltdest. Wasser auf 100 ccm gebracht.

Der Pilzertrag in Prozentsatz zu der auf gewöhnlichem Kulturmedium (NH_4NO_3 als N-Quelle) ist in der Tabelle XXXVII und Fig. 5 angegeben.

TABELLE XXXVII.

KNO_3-Zusatz				$(NH_4)_2HPO$-Zusatz			
Menge der zugesetzten Rosahefe	Erntegew. mg	pH	Verhältnis	Menge der zugesetzten Rosahefe	Erntegew. mg	pH	Verhältnis
Anfangs-pH	6.2			6.4			
0	129.5	6.3	100.0	Kontrolle	165.0	3.0	100.0
	99.0	6.2			220.5	3.2	
	133.5	6.2			210.5	2.9	
$1/2 \times 10^{-4}$	82.0	6.2	72.4	$1/7 \times 10^{-4}$	112.0	4.8	59.4
	102.0	6.2			113.0	4.8	
	78.0	6.2			120.0	4.2	
$1/4 \times 10^{-4}$	91.0	6.2	73.7	$1/9 \times 10^{-4}$	108.5	5.0	60.6
	94.0	6.2			121.0	3.9	
	82.0	6.2			123.0	3.9	
$1/8 \times 10^{-4}$	84.5	6.2	76.2	$1/14 \times 10^{-4}$	98.0	4.2	54.1
	89.0	6.2			110.5	4.2	
	102.0	6.2			106.0	4.3	
$1/12 \times 10^{-4}$	87.0	6.2	76.8	$1/16 \times 10^{-4}$	106.0	4.2	55.6
	90.0	6.2			106.0	4.2	
	101.0	6.2			111.5	4.2	
$1/16 \times 10^{-4}$	106.0	6.2	96.8	$1/24 \times 10^{-4}$	99.5	4.2	51.6
	120.0	6.2			100.0	4.2	
	124.5	6.2			100.5	4.2	
$1/32 \times 10^{-4}$	132.0	6.2	102.1	$1/32 \times 10^{-4}$	162.5	3.4	80.0
	132.5	6.2			166.0	3.5	
	105.0	6.2			141.5	3.7	
$1/64 \times 10^{-4}$	125.0	6.3	106.4	$1/64 \times 10^{-4}$	158.0	3.1	79.6
	135.0	6.3			169.5	2.8	
	125.0	6.3			135.0	3.4	
$1/128 \times 10^{-4}$	170.0	6.3	131.1	$1/128 \times 10^{-4}$	170.5	2.8	106.0
	171.5	6.3			188.0	2.9	
	133.0	6.3			226.0	2.4	
				$1/256 \times 10^{-4}$	224.0	2.3	97.5
					174.0	2.6	
					168.5	2.8	

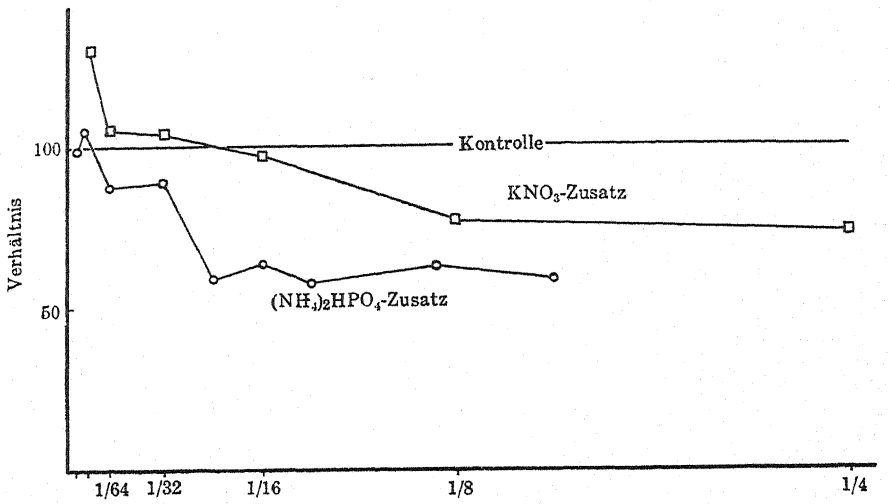


Fig. 5.

Wie man sieht, gestaltet sich die Empfindlichkeit des Pilzes gegen die Giftsubstanz bei diesen zwei Kulturreihen nicht ganz gleich, indem z.B. der Schwellenwert der Rosahefemenge, der eine eben noch feststellbare Wachstumshemmung bewirkt, bei Nitrat-Zusatz 1/160000 (0.000625 %) bei Ammonium-Zusatz dagegen 1/640000 (0.00015625 %) beträgt.

(3) pH-Abhängigkeit

Versuch XXIV

Es wurde weiter untersucht, wie sich die Wirkung der Giftsubstanz bei verschiedenem pH-Wert äussert. Zur Anwendung kamen folgende Nährlösungen.

Versuchsreihe	1	2	3	4	5	6
Rohrzucker (M/2) . . .	50 ccm	50 ccm	50 ccm	50 ccm	50 ccm	50 ccm
H_3PO_4 (M/2)	1.9 ,,					
KH_2PO_4 (M/2)	23.1 ,,	24.2 ,,	23.5 ,,	20.0 ,,	7.5 ,,	
K_2HPO_4 (M/2)		0.8 ,,	1.5 ,,	5.0 ,,	17.5 ,,	17.0 ,,
K_3PO_4 (M/2).						8.0 ,,
Lösung A	20 ,,	20 ,,	20 ,,	20 ,,	20 ,,	20 ,,
Rosahefelösung . . .	5 ,,	5 ,,	5 ,,	5 ,,	5 ,,	5 ,,
Summe	100 ,,	100 ,,	100 ,,	100 ,,	100 ,,	100 ,,
Anfangs-pH	2.7	3.8	4.4	5.6	6.5	7.5
Anfangs-Titrations-acidität (N/10 NaOH ccm)	15.00	12.90	12.66	11.34	8.18	1.91

Kulturdauer: 4 Tage bei 27°.

Die Versuchsresultate sind in Tabelle XXXVIII und Fig. 6 angegeben.

In Übereinstimmung mit den früheren Befunden TAMIYAS zeigt die pH-Wachstumskurve zwei Maxima, wobei aber das eine an Säureseite (pH 4.4) etwas höher liegt als dasjenige an Alkaliseite (pH 7.5). Die Kurve der mit Rosahefe versetzten Kultur läuft stets ungefähr parallel mit der der Kontrollkultur und zwar erleidet das Pilzwachstum im geprüften pH-Bereich von 2.7 bis 7.5 überall um etwa gleichen Betrag die Wirkung der Giftsubstanz, eine Tatsache, die von der von TAMIYA bei Zusatz von verschiedenen Metall- oder Säureionen beobachteten verschieden ist.

TABELLE XXXVIII.

Menge der zugesetzten Rosahefe	Erntegew. mg	Kojisäure %	Kojisäure-produktiv.	Titrationsacidität ccm	pH
				(Anfang) 15.00	(Anfang) 2.7
Ohne Zusatz	88.5	0.49	1.41	18.56	2.6
	69.0	0.35	1.26	17.65	2.6
	96.0	0.42	1.09	18.56	2.6
1/20000	45.0	0.18	1.00	16.56	2.6
	36.0	0.12	0.84	15.80	2.6
	38.0	0.12	0.79	15.76	2.7
1/160000	48.5	0.15	0.77	16.09	2.6
	33.0	0.15	1.14	15.82	2.6
	38.5	0.12	0.79	15.76	2.5
1/320000	62.0	0.35	1.41	17.91	2.6
	38.0	0.15	0.96	16.22	2.6
	33.0	0.15	1.14	15.91	2.6
1/1280000	95.0	0.48	1.26	19.38	2.5
	90.0	0.64	1.78	19.77	2.5
	92.0	0.56	1.52	19.51	2.5
				(Anfang) 12.90	(Anfang) 3.8
Ohne Zusatz	165.0	0.42	0.63	17.38	2.8
	174.5	0.63	0.90	18.45	2.8
	192.0	0.63	0.82	18.49	2.7
1/20000	108.5	0.49	1.13	17.29	2.7
	125.0	0.45	0.90	18.45	2.6
	145.0	0.54	0.93	18.49	2.6
1/160000	113.5	0.56	1.23	18.13	2.7
	122.0	0.42	0.87	17.35	2.7
	158.5	0.63	0.99	18.87	2.6

TABELLE XXXVIII.

(Fortsetzung 1)

Menge der zugesetzten Rosahefe	Erntegew. mg.	Kojisäure %	Kojisäure-produktiv.	Titrations-acidität ccm	pH
				(Anfang) 12.90	(Anfang) 3.8
$\frac{1}{320000}$	122.5	0.63	1.28	18.31	2.5
	122.5	0.56	1.14	18.87	2.5
	155.0	0.72	1.16	18.49	2.8
$\frac{1}{1280000}$	205.0	0.72	0.88	19.24	2.8
	171.5	0.72	1.05	18.35	2.8
	166.0	0.88	1.34	19.20	2.6
				(Anfang) 12.66	(Anfang) 4.4
Ohne Zusatz	163.0	0.77	1.18	18.49	2.7
	216.5	0.90	1.04	18.20	2.6
	161.0	0.60	0.93	17.47	2.6
$\frac{1}{20000}$	121.5	0.58	1.19	18.38	2.7
	128.0	0.55	1.08	18.39	2.6
	161.0	0.60	0.93	17.84	2.7
$\frac{1}{160000}$	117.5	0.56	1.19	17.47	2.6
	147.0	0.62	1.05	18.58	2.6
	128.0	0.50	0.98	17.35	2.7
$\frac{1}{320000}$	179.0	0.50	0.70	17.13	2.8
	138.5	0.62	1.12	18.40	2.6
	174.0	0.50	0.72	16.93	2.7
$\frac{1}{1280000}$	193.0	0.60	0.70	17.82	2.8
	164.0	0.58	0.88	17.45	2.8
	204.5	0.50	0.61	17.07	2.8

Tabelle XXXVIII.

(Fortsetzung 2)

Meng der zugesetzten Rosahefe	Erntegew. mg	Kojisäure %	Kojisäure-produktiv.	Titrations-acidität ccm	pH
				(Anfang) 11.34	(Anfang) 5.6
Ohne Zusatz	110.5	0.30	0.68	14.43	3.7
	120.0	0.30	0.62	14.33	3.6
	107.5	0.28	0.65	14.38	3.6
$\frac{1}{20000}$	88.5	0.25	0.71	14.72	3.7
	68.5	0.23	0.84	14.21	3.7
	84.5	0.24	0.71	14.54	3.6
$\frac{1}{160000}$	81.0	0.25	0.77	13.47	3.6
	78.0	0.18	0.58	13.83	3.7
	84.0	0.20	0.60	14.41	3.6
$\frac{1}{320000}$	107.0	0.22	0.51	13.74	3.6
	106.5	0.30	0.71	14.72	3.5
	118.0	0.18	0.38	13.92	3.6
$\frac{1}{1280000}$	101.0	0.30	0.74	13.21	3.6
	108.0	0.22	0.51	13.98	3.6
	107.0	0.23	0.54	13.74	3.6
				(Anfang) 8.18	(Anfang) 6.5
Ohne Zusatz	95.0	0.20	0.52	11.54	5.4
	100.0	0.25	0.63	12.18	5.4
	85.0	0.15	0.44	11.12	5.5
$\frac{1}{20000}$	78.0	0.20	0.64	11.63	5.6
	69.0	0.12	0.43	11.03	5.6
	69.5	0.10	0.36	10.90	5.6
$\frac{1}{160000}$	101.0	0.12	0.30	11.01	5.6
	71.5	0.14	0.49	11.25	5.6
	64.0	0.10	0.39	10.87	5.6

TABELLE XXXVIII.

(Fortsetzung 3)

Menge der zugesetzten Rosahefe	Erntegew. mg.	Kojisäure %	Kojisäure-produktiv.	Titrations-acidität ccm	pH
				(Anfang) 8.18	(Anfang) 6.5
$\frac{1}{320000}$	76.0	0.10	0.33	10.90	5.5
	73.0	0.12	0.41	10.54	5.7
	79.0	0.14	0.44	11.23	5.5
$\frac{1}{1280000}$	67.5	0.14	0.52	11.30	5.6
	79.0	0.18	0.57	11.27	5.5
	99.0	0.21	0.51	11.47	5.4
				(Anfang) 1.91	(Anfang) 7.5
Ohne Zusatz	119.0	0.60	1.26	9.56	6.4
	90.5	0.48	1.32	8.66	6.4
	102.5	0.50	1.00	8.92	6.4
$\frac{1}{20000}$	59.0	0.32	1.35	7.44	6.6
	66.5	0.25	0.94	6.37	6.6
	80.5	0.42	1.34	7.46	6.0
$\frac{1}{160000}$	70.0	0.48	1.67	7.64	6.5
	55.0	0.36	1.64	8.01	6.6
	52.5	0.28	1.33	6.37	6.6
$\frac{1}{320000}$	95.0	0.40	1.05	7.37	6.6
	84.0	0.42	1.25	7.37	6.6
	66.0	0.38	1.44	7.30	6.6
$\frac{1}{1280000}$	78.5	0.50	1.59	8.48	6.5
	120.5	0.50	1.04	9.26	6.5
	109.5	0.60	1.37	10.28	6.4

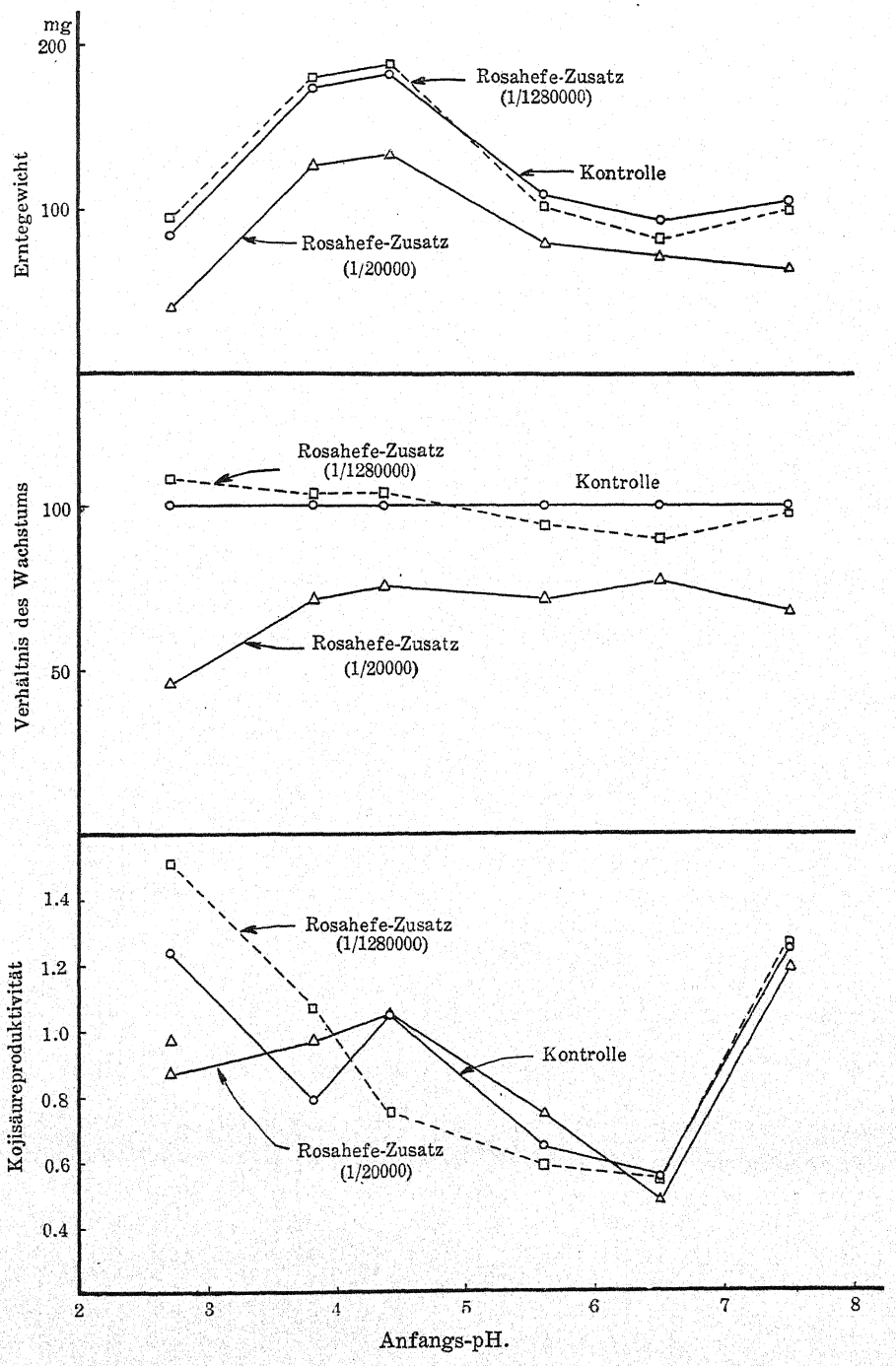

mg
200
100
Erntegewicht
Rosahefe-Zusatz
(1/1280000)
Kontrolle
Rosahefe-Zusatz
(1/20000)
Verhältnis des Wachstums
100
50
Rosahefe-Zusatz
(1/1280000)
Kontrolle
Rosahefe-Zusatz
(1/20000)
Kojisäureproduktivität
1.4
1.2
1.0
0.8
0.6
0.4
Rosahefe-Zusatz
(1/1280000)
Kontrolle
Rosahefe-Zusatz
(1/20000)
2
3
4
5
6
7
8
Anfangs-pH.

Fig. 6.

(4) Gärungsversuche

Versuch XXV

Der Einfluss der von unserer Rosahefe gebildeten Substanz auf die alkoholische Gärung wurde an Hand der Bierhefe und *Asp. oryzae* geprüft. Eine 1%-ige Bierhefesuspension in gewöhnlicher rohrzuckerhaltiger Kulturlösung (pH 4.0) wurde mit verschiedenen Mengen der abgetöteten Rosahefezellen versetzt und das Gärungsvermögen unter Anwendung von Gärungssaccharometer TAMIYAS[1] verglichen. Die Gärungstemperatur war 23°.

TABELLE XXXIX.

Zeit Min.	Kontrolle			Rosahefe-Zusatz					
				1/10000 (0.01%)			1/100000 (0.001%)		
60	+	+	+	+	+	+	+	+	+
110	4.0	6.7	2.2	5.7	5.1	10.3	5.5	8.4	7.3
120	6.4	11.1	3.9	8.4	8.3	14.4	8.6	12.4	9.8
130	12.4	14.4	7.9	12.1	12.0	18.7	12.9	16.8	13.1
140	18.1	18.9	14.0	16.4	16.1	21.6	17.4	20.3	17.1
150	22.2	23.3	19.2	25.4	20.6	27.1	25.6	26.5	25.0
160	25.5	25.7	20.6	29.5	23.1	30.2	28.6	29.2	27.2
170	30.2	34.5	25.7	34.3	27.1	37.8	34.0	35.9	31.7
180	33.8	38.5	30.2	37.0	29.6	40.7	39.0	39.0	34.4
Summe	102.5 ccm			107.3 ccm			112.4 ccm		

2 g (Trockengewicht) Rosahefe wurden weiter mit 200 ccm von 0.1% HCl 20 Stunden lang bei 30° extrahiert. Von dieser Lösung wurden folgende drei Portionen zubereitet:

(a) Filtrat durch SEITZ-Filter.
(b) Filtrat durch gewöhnliches Filtrierpapier.
(c) Ohne Filtrierung.

Je 40 ccm von diesen drei Portionen wurden mit Rohrzucker und gewöhnlichen Nährsalzen versetzt und mit dest. Wasser auf 100 ccm gebracht (Rohrzuckergehalt 8.6%, pH 3.8). Unter Zusatz der Bierhefe (0.5%) zu diesen drei Ansätzen erhielt man die Resultate, die in der Tabelle XL angegeben sind. (Gärungstemperatur: 16°.)

(1) H. TAMIYA: Acta Phytochimica, **4**, 1928, 87.

TABELLE XL

Zeit Min.	Filtrat durch SEITZ-Filter			Filtrat durch Filtrierpapier			Kontrolle		
180	4.0	2.4	3.2	2.2	3.2	2.4	2.4	2.0	2.8
240	10.8	8.7	8.7	9.8	12.0	12.8	10.2	8.5	7.0
300	16.2	15.6	18.0	15.4	17.0	19.2	15.6	17.2	13.6
360	20.0	20.0	21.0	18.0	19.2	21.3	17.9	20.3	17.0
Summe	61.0 ccm			58.4 ccm			55.3 ccm		

Wie aus Tabelle XXXIX und XL ersichtlich, wirkt die von Rosahefe gebildete Substanz, im Gegensatz zu der von HAYDUCK[1] bei gewissen Hefearten beobachteten, gar nicht hemmend auf die Hefegärung.

Versuch XXVI

Die Beeinflussbarkeit der anaeroben Gärung von *Aspergillus oryzae* wurde mit der von TAMIYA[2] vorgeschlagenen Methode probiert. Alter der Pilzdecke: 3 Tage. Gärungstemperatur: 30°.

TABELLE XLI

Gärungsdauer (Tage)	1	2	4	5	6	Pilzgew. mg	$\left(\frac{CO_2(ccm)}{Pilzgewicht\ (g)}\right)$
Kontrolle	6.3	15.0	34.0	37.0	43.8	62.0	706.5
	4.4	7.8	28.4	34.8	47.8	58.0	824.2
	5.8	14.0	32.7	36.5	42.1	55.5	758.6
	2.8	11.0	25.8	46.0	51.8	48.0	1079.2
Vorkultur: ohne Zusatz; Gärungsmedium: Rosahefe-Zusatz	2.0	6.6	23.2	26.6	34.5	59.0	584.7
	5.0	19.4	43.5	49.1	60.1	82.5	728.5
	6.4	22.0	45.8	48.8	55.6	78.5	708.3
	2.0	7.0	25.8	30.8	42.8	49.5	864.6
Vorkultur und Gärungsmedium: Rosahefe-Zusatz	2.0	3.2	8.8	10.8	14.4	30.0	480.0
	1.3	3.1	14.2	17.2	26.8	41.0	653.7
	0.8	2.3	7.8	9.0	12.0	26.0	461.6
	2.2	6.0	20.2	25.3	34.7	39.5	878.5

(1) F. HAYDUCK: loc. cit.
(2) H. TAMIYA: Acta Phitochimica, **4**, 1928, 152.

Aus Tabelle XLI ist zu entnehmen, dass der von Rosahefe gebildete Stoff sowohl bei Zusatz zu der Vorkultur von *Asp. oryzae* wie auch bei Zugabe zu Gärungsmedium auf Gärungsumsatz nicht wesentlich einflussgebend ist.

Schlussbemerkungen

Die oben dargestellen experimentellen Befunde geben uns ein klares Bild dafür, dass die von mir untersuchte Rosahefeart (*Torula Suganii*) zweierlei Substanzen oder Substanzkomplexe bildet, wovon die eine auf Schimmelpilzwachstum schädigend und die andere auf dasselbe befördernd wirkt. Die in Betracht kommende Giftsubstanz, die wir hier der Kürze halber als X-Substanz bezeichnen wollen, unterscheidet sich nun von den ähnlichen, bisher von einigen Forschern angegebenen Giftsubstanzen in Hefe- oder Schimmelpilzzellen in mehreren Punkten. Angeführt sei hier zunächst folgendes :

(1) Die X-Substanz ist kochbeständig, nicht flüchtig und wird durch Alkali (aber nicht durch Säure) zerstört, während die von FERNBACH und VULQUIN untersuchte Giftsubstanz in gewissen Hefearten kochlabil, flüchtig und gegen Alkali widerstandsfähiger, gegen Säure aber minder widerständsfähig zu sein scheint. Übrigens wird die von den genannten Forschern angegebene Substanz durch Pepsin oder Trypsin und auch durch Zusatz von Kalk inaktiviert wird.

(2) Das Wachstum und die Gärung der Hefe wird durch Zusatz von X-Substanz nicht gehemmt, wohl aber bei Zusatz von Hefeextrakt nach HAYDUCK und FERNBACH und VULQUIN.

(3) Die neuerdings von SATOH entdeckte Giftsubstanz in *Ophiobolus Miyabeanus* stimmt insofern mit unserer X-Substanz überein, als sie wasserlöslich ist und für Schimmelpilzwachstum hemmend wirkt, weicht aber von unserer Substanz darin ab, dass sie kochlabil und nicht nur auf das Wachstum der Pilzmyzelien sondern auch auf die Sporenkeimung einflussgebend ist.

Was nun die Wirkungsweise der wachstumsbefördernden Substanz in Rosahefezellen anlangt, so stossen wir nicht selten auf ähnliche Beispiele in Angaben verschiedener Forscher. Neuerdings haben TAKATA und auch TAKAHASHI und LIN angegeben, dass das Wachstum von *Asp. oryzae* durch Zusatz von Vitamin B oder Bios merklich beschleunigt wird. Auch SATOH hat kürzlich nachgewiesen, dass *Ophiobolus Miyabeanus* neben der auf Schimmelpilzwachstum hemmend

wirkenden Substanz einen auf demselben reizend wirkenden Stoff bildet, der durch Filtrierung durch CHAMBERLAND-Filter vom ersteren getrennt werden kann.

Seitdem WILDIERS klargelegt hat, dass die Bierhefe kaum in einer rein synthetischen Zucker-Salz-Nährlösung, wohl aber bei Zusatz geringfügiger Menge von Würze oder Hefewasser vermehren kann, wurden die Eigenschaften von dieser wachstumsbefördernden Substanz vielfach untersucht. Zwischen der in der vorliegenden Arbeit nachgewiesenen wachstumsfördernden Substanz in Rosa- und Bierhefe, die wir hier als Y-Substanz bezeichnen wollen, und der von WILDIERS entdeckten und von ihm als Bios bezeichneten Substanz finden wir wohl folgende Übereinstimmung in ihren Eigenschaften:

(1) Sie sind kochstabil. (Der von SATOH bei *Ophiobolus Miyabeanus* nachgewiesene wachstumsbefördernde Stoff ist auch kochstabil.)

(2) Sie sind ebenfalls löslich in Wasser, nicht aber in Äther, Aceton und absolutem Alkohol.

(3) Sie werden in Hefeasche nicht nachgewiesen.

Die Frage, ob unsere Y-Substanz nichts anders sei als Bios selbst, kann man an Hand des vorliegenden Tatsachenmaterials nicht näher diskutieren. Jedenfalls dürfte man aber wohl mit Recht behaupten, dass von den Rosahefezellen zweierlei Substanzen mit den zueinander ganz entgegengesetzten physiologischen Wirkungen gebildet werden, so dass bei gleichzeitiger Zugabe dieser beiden Substanzen als Endeffekt wohl die Differenz der Wirksamkeit dieser beiden Faktoren zum Vorschein kommt. Andererseits liegt es nahe anzunehmen, dass die relative Empfindlichkeit des Zellwachstums gegenüber beiden in Frage kommenden Substanzen je nach der Organismenarten weitgehend verschieden sein kann. Man könnte also sagen, dass der beobachtete Unterschied in der Widerstandsfähigkeit von Hefen und Schimmelpilzen gegen den Rosahefezusatz nicht um eine qualitative sondern nur um eine quantitative Verschiedenheit der Empfindlichkeit der Organismen gegeüber X- bzw. Y-Substanz handle. Diese Annahme gewinnt um so an Wahrscheinlichkeit, wenn man daran denkt, dass alle zu meinen Versuchen angewandten Hefearten, die bei Zusatz der Rosahefezellen keine Wachstumshemmung erleiden, für ihr Wachstum unbedingt das Vorhandensein der biosartigen Substanz nötig haben und deren Wachstum durch eine sehr geringfügige Menge von dieser Substanz erheblich gesteigert wird. Als weitere Stütze für diese Annahme sei noch erwähnt, dass *Rhizopus nigricans*, dessen Wachstum im Vergleich

mit dem von *Asp. oryzae* durch biosartige Substanz viel deutlicher gesteigert wird, durch Zusatz von abgetöteten Rosahefezellen nicht so sehr Wachstumshemmung erleidet wie *Aspergillus*.

Zusammenfassung

(1) Es wurde festgestellt, dass die Kultur einer Rosahefeart (*Torula Suganii*) sowohl frisch als auch nach dem Abkochen auf das Wachstum verschiedener Schimmelpilze, nicht aber auf das von Hefen, hemmend wirkt.

(2) Die Giftwirkung der Rosahefesubstanz erstreckt sich nicht auf die Keimungsfähigkeit der Konidien oder auf das Wachstum der erwachsenen Pilzdecke, sondern überhaupt nur auf das Wachstum der ganz jüngen Pilzmyzelien.

(3) Die alkoholische Gärung von Bierhefe und *Asp. oryzae* wird durch die in Frage kommende Giftsubstanz nicht gehemmt.

(4) Neben der auf Pilzwachstum giftig wirkenden Substanz kommt in der Rosahefe auch ein Stoff vor, der auf Pilzwachstum befördernd wirkt. Dieser Stoff scheint bei gleichzeitigem Zusatz mit Giftsubstanz gegenüber der letzteren das Gegengewicht zu halten, sodass bei längerer Kulturdauer, wo die Empfindlichkeit des Pilzes gegen die Giftsubstanz verschwunden ist, nur die wachstumsreizende Wirkung zu Tage tritt.

(5) In der Kultur von Bierhefe sowie von einigen Rosahefearten wurde das Vorhandensein der auf Schimmelpilzwachstum beschleunigend wirkenden Substanz, nicht aber der Giftsubstanz nachgewiesen.

(6) Die Wirkung dieser Giftsubstanz auf das Schimmelpilzwachstum lässt sich schon bei ganz geringfügigem Zusatz (z.B. 1 mg Rosahefezellen pro 100 ccm Kulturlösung) deutlich erkennen. Die minimale Konzentration der Rosahefezellenzusatz für erkennbare Giftwirkung fällt bei NH_4NO_3- oder KNO_3-Zusatz etwas grösser aus als bei Zusatz von $(NH_4)_2HPO_4$ als N-Quelle für Schimmelpilzkultur. Der Grad der Giftwirkung dieser Substanz wird von der Acidität der Kulturlösung des Schimmelpilzes im Bereich von pH 2.7 bis 7.5 nicht wesentlich modifiziert.

(7) Die Giftsubstanz ist kochbeständig, widerstandsfähig gegen ultraviolette Strahlen, gut löslich in heissem Aceton, Äthylalkohol,

Äther und Chloroform, aber sehr wenig in CS_2, Benzol und Petroläther. Von Filtrierpapier, Kaolin und Pilzmyzelien wird sie sowohl in saurer als auch in schwach alkalischer Reaktion stark adsorbiert, und die an Kaolin oder Filtrierpapier adsorbierte Giftsubstanz lässt sich mit Äther gut extrahieren. Durch stark alkalische Reaktion (pH 9.6) wird die Wirkung der Giftsubstanz irreversibel zerstört. Die Aschenbestandteile der Rosahefezellen haben mit der Giftwirkung derselben nichts zu tun.

(8) Der mit wirksamen Lösungsmitteln (Aceton, Äthylalkohol, Äther und Chloroform) extrahierte Rückstand der Rosahefezellen zeigt keine Giftwirkung mehr, während in demselben die auf Schimmelpilzwachstum reizend wirkende Substanz zurückbleibt. Auch die Rückstände der Petroläther-, Benzol- oder CS_2-Auszüge der Rosahefezellen wirken beschleunigend auf das Wachstum von *Asp. oryzae*, wenn sie nachträglich mit Aceton extrahiert werden. Acetonauszug der Bierhefe ist wirkungslos auf das Schimmelpilzwachstum, während ihr Rückstand darauf kräftig reizend wirkt.

(9) Die wachstumsbeschleunigende Substanz in Rosahefe ist beständig gegen ultraviolette Strahlen, nicht löslich in Äther und absolutem Alkohol und wird von Kaolin oder SEITZ-Filter nicht adsorbiert. Durch Behandlung mit Äther, Kaolin oder SEITZ-Filter können also die Gift- und Reizsubstanz leicht von einander getrennt werden.

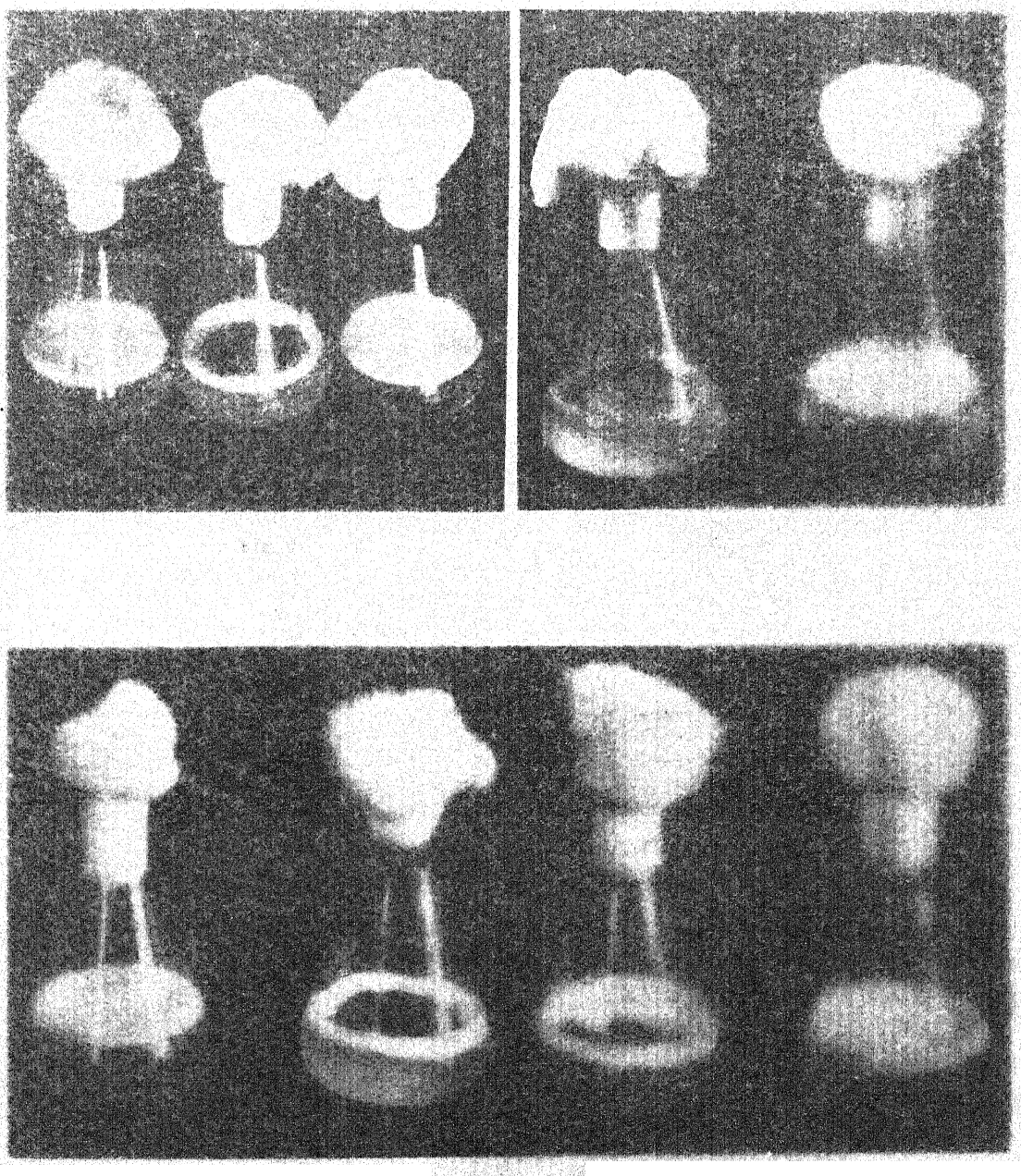
Meinem verehrten Lehrer, Herrn Professor Dr. Keita SHIBATA, der mich zu dieser Arbeit anregte, möchte ich für seine liebenswürdige Leitung und Anteilnahme bei der Ausführung dieser Untersuchungen meinen herzlichsten Dank aussagen. Ferner bin ich auch Herrn Dr. Hiroshi TAMIYA für seine gütige Unterstützung bei dieser Arbeit zum besten Dank verpflichtet.

Erklärung von Tafel IX

Fig. 1. *Aspergillus oryzae:* Kulturdauer 4 Tage bei 30°C., von links: Kontrolle, Rosahefe-Zusatz, Bierhefe-Zusatz.

Fig. 2. *Rhizopus nigricans*: Kulturdauer 2 Tage bei 25°C., links Rosahefe-Zusatz, rechts Kontrolle.

Fig. 3. Kultur von *Aspergillus oryzae* bei Zusatz der Rosahefe in verschiedenen Mengen, von links: Kontrolle, 1×10^{-4}-, $1/32\times10^{-4}$-, $1/256\times10^{-4}$-Zusatz.



Abstracts Nos. 292–388

(Referring to the principal papers on Botany and allied subjects which have appeared in Japan chiefly during January – June 1931)

292. Species Novae Caricum Japonicarum. Shigeo AKIYAMA. (Jour. Fac. Sc., Hokkaido Imp. Univ. Ser. V (Bot.) **1**, 1931, 57–63, 9 pls.).

The following new species are described and illustrated: *Carex kasugayamensis*, *C. grandilimosa*, *C. scabro-aristata*, *C. Doiana*, *C. squamoidea*, *C. gagaensis*, *C. yezo-montana*, *C. vulpo-caudata*. Some new varieties are also described.

293. Die Myxomyceten der Südmandschurei. (Mit japan. Zfg.). Yoshikadzu EMOTO. (Bot. Mag. Tôkyô **45**, 1931, 229–233, 3 Textfig.).

Der Verf. hat eine Anzahl von Myxomyceten aus Südmandschurei enumeriert, unter denen eine Art, *Physarum puniceum* neu ist und ausführlich beschrieben und abgebildet wird.

294. Comparative Studies on the Morphology and Physiology of Japanese and Philippine *Hypochnus* as well as *Hypochnus Solani*. (Japanese). Sigeru ENDÔ. (Agric. Studies **14**, 1930, 3 pp.).

The author has made formerly the statement that *Hypochnus Sasakii* from Japan is identical with the fungus from the Philippines which PALO calls that of the *Rhizoctonia Solani* group (cf. Japan. Jour. Bot. **3**, Abst. No. 220). In consequence of his comparative experiments of these fungi in reference to their form, disease spots produced by their artificial infection, temperature relation (cf. the next No.) the author could confirm his former statement. *Rhizoctonia Solani* (*Corticium vagum* or *Hypochnus Solani*) widely distributed in Europe and America was found to differ from the above in various respects.

295. On the Influence of the Temperature upon the Development of *Hypochnus*. (Japanese). Sigeru ENDÔ. (Ann. Phytopathol. Soc. Japan **2**, 1930, 1–3).

The temperature relation of Japanese *Hypochnus Sasakii* and the Philippine species in respect to the infection of rice plants was studied. By placing plants artificially infected with respective sclerotia under the temperatures varying from 24–36°C it was found that the infection takes place most rapidly under 32°C (18–24 hrs.), under 28°C only after 24 hrs., and under 24° and 36° no infection even after 24 hrs. Furthermore, to study the intensity of development of the fungi after infection at various temperatures the author has calculated the number of disease spots every day after infection; it was found that their development is most vigorous when infected at 28° and 32°C, and especially the latter. The above noted relation between the temperature and the fungus may be explained in virtue of the author's

observation that the formation of hyphae from the sclerotia takes place best at 28-32°C.

296. Studies on the Antagonism of Microorganisms. I. Growth of *Hypochnus centrifugus* TUL. as Influenced by the Antagonistic Action of Other Microorganisms. (With Japanese résumé). Sigeru ENDÔ. (Bull. Miyazaki Coll. Agric. & For. **3**, 1931, 95–119, 1 pl.).

For experiments noted in the above title the author has employed beside *Hypochnus centrifugus* 26 species of bacteria and 62 species of fungi. First of all, any one of 26 species of bacteria just noticed and a sclerotium of *Hypochnus* were inoculated on a certain culture medium, either both at the same time or one after another (i.e. the bacteria after a certain development of *Hypochnus*). It was then observed that certain species of bacteria cover *Hypochnus* to prevent entirely or at least partially its growth, leading finally to the death of sclerotia. Certain other species are much more tolerant in their antagonistic behaviour against *Hypochnus*, inasmuch as the growth of both organisms are seen to stop, leaving a certain interval between them. Still other species of bacteria are quite indifferent towards the growth of *Hypochnus*. Similar experiments were performed with *Hypochnus* on one hand, and several species of fungi, as *Aspergillus*, *Penicillium*, *Mucor* and *Absidia* on the other. It was found that generally the latter fungi are covered by the mycelia of *Hypochnus*, except a few which retard the growth of the latter to a certain extent.

297. On the Short Day and Illumination Treatment in Rice, referring specially to the Time and Duration of Treatment. (Japanese with English résumé). Yutaka FUKE. (Jour. Imp. Agric. Exp. Sta. **1**, 1931, 263–286, 3 figs.).

Experiment for changing the heading time of various races of rice plants by short day method and illumination treatment are described.

In short day method the plants were exposed to daylight from 8 a.m. to 4 p.m., and the rest of the day placed in darkness. In general, the earlier in the plant's development the treatment commences, the earlier sets in the heading time. So that for instance when the treatment was begun at the stage in which the plant bears 7–9 leaves and the tillering begins to become frequent, it was much more efficacious for accelerating the heading time than when it was first begun 30 days prior to the natural heading.

By illuminating rice plants by electric light during the night the heading is delayed, when this treatment commences about 40 days prior to the natural heading time.

298. On the Mosaic Disease of Broad Beans. (Japanese). Teikichi FUKUSHI. (Jour. Plant Prot. **17**, 1930, 707–712, 779–784, 1 pl.).

An account is given of the host range, symptoms, cause, and the modes of transmission of the mosaic disease of broad beans which is prevalent in the Prefecture of Tottori. Author.

299. On the Modes of Transmission of the Mosaic Disease of Tobacco. (Japanese with English résumé). Teikichi FUKUSHI. (Jour. Sapporo Soc. Agric. & For. **22**, 1931, 305–320).

The author discusses various modes of transmission of mosaic disease of tobacco hitherto proposed, and he thinks the following as the only possible way. In Japan tobacco growers are accustomed to smoke the pipe tobacco (or cut tobacco), and it may be expected that the fingers become easily contaminated with the virus during smoking it, if it carries the virus of the mosaic disease. It is highly probable that tobacco growers may spread the disease in the operation of transplanting, topping and weeding of the tobacco plant with hands which have been contaminated with the virus during smoking the pipe tobacco. The results of infection experiments making the above supposition very probable are mentioned.

300. Angular Spot of *Zinnia elegans* JACQ. Caused by Eelworms. (Preliminary Report) (With Japanese résumé). Teikichi FUKUSHI and Hidesaku SAITO. (Trans. Tottori Soc. Agric. & For. **2**, 1930, 45–56, 1 pl.)

Infection experiments have shown that the disease is due to the attack of a nematode, *Aphelenchus Ritzerma Bosi* SCHWARTZ.

301. Formation of Diploid and Tetraploid Gametes in *Brassica*. Eiji FUKUSHIMA. (Japan. Jour. Bot. **5**, 1931, 273–283, 1 pl. and 12 text-figs.).

302. Studies on Septorioses of Plants II. *Septoria Azaleae* VOGLINO Causing the Brown-spot Disease of the Cultivated Azaleas in Japan. Takewo HEMMI and Shizuko KURATA. (Mem. Coll. Agric., Kyoto Imp. Univ. No. **13**, 1931, 22 pp., 2 pls. and 4 text-figs.).

A serious disease of cultivated Azaleas (as *Rhododendron ledifolium, obtusum,* etc.) is widely spread near Kyoto. This is due to *Septoria Azaleae* VOGLINO according to the authors. This disease appears first in early autumn on the leaves, commonly as brown spots, and gradually spreading, leads to the loss of many leaves, and consequently to extreme weakness of the host. The authors discuss in this paper the morphology and the cultural characters of the causal organism. It grows best at 16–28°C, and its greatest expansion takes place at 24°C. Experimental inoculation by conidia was successful. In these experiments it was further found that the incubation period of the disease endures as long as two months.

303. *Cintractia Machili* n. sp., a New Smut of *Machilus longifolia* BLUME and *M. Thunbergii* SIEB. et ZUCC. var. *glauscescens* BLUME. (With Japanese résumè). Iwao HINO and Eiri NAGAOKA. (Bull. Miyazaki Coll. Agric. & For. No. **3**, 1931, 49–58, 2 pls. and 3 text-figs.).

Cintractia Machili is a new species of smut-fungi found on the diseased winter-buds of *Machilus longifolia* and *M. Thunbergii* var. *glauscescens*. It was formerly called either *Ustilago* sp. or *Anthracoidea Onumae* by certain authors, but it is decidedly different from either in various respects.

304. On the Gaseous Exchange in *Synedra* sp. Keinosuke HIRAMATSU. (Sc. Rpts. Tôhoku Imp. Univ. Ser. IV, **6**, 1931, 237–250, 6 text-figs.).

The author's methods for studying the gaseous exchange in a marine diatom *Synedra* sp. are first described in detail. The results derived from his studies are

summarized as follows. The CO_2-assimilation takes place more intensely in more concentrated CO_2-source than in dilute one. Though within the range of small light intensities the assimilation curve is almost linear, the assimilation rate becomes smaller under strong light. The assimilation process and the temperature increase proportionally till the point when light comes to limit the process.

305. On the Microcyclic Species of the Pucciniaceae Collected in Some Mountains in Japan. (Japanese). Naohide HIRATSUKA. (Trans. Tottori Soc. Agric. Sc. **3**, 1931, 211–253, 1 pl. and 1 fig.).

The paper contains the results of studies on the microcyclic species of the Pucciniaceae found in various high mountains of Northern and Middle Japan, incl. Saghalien and Hokkaido, varying in their height from 500 to almost 2900 m above sea-level. The fungi belong to 6 genera and 45 species, of which 29 are micro- and 16 leptosporic. The general conclusion is that the microsporic species increase gradually from the foot towards the summit of the mountain.

A new species *Puccinia hakkodense* HIRATSUKA f. is described.

306. Studies on Septorioses of Plants. IV. New or Noteworthy Species of *Septoria* Found in Japan. Shigekatsu HIRAYAMA. (Mem. Coll. Agric., Kyoto Imp. Univ. No. **13**, 1931, 33–40, 2 pls. and 4 text-figs.).

10 species of *Septoria* are enumerated. The following are new: *S. Abeliceae*, *S. Astroemoea Savatierii*, *S. Ecliptae* and *S. Glechomae*.

307. Observations and Experiments on the Mulberry Rust Caused by *Aecidium Mori* BARCLAY. Makoto HIURA. (Japan. Jour. Bot. **5**, 1931, 253–272, 3 pls. and 2 text-figs.).

308. Nuntia ad Floram Japonicam IX, X, XI. (With Japanese résumé). Masaji HONDA. (Bot. Mag. Tôkyô **45**, 1931, 1–7, 26–27, 43–45, 71–72, 138–139, 161–162).

The following new species are described among others: *Arabis dentipetalata*, *Krascheninikowia ciliata*, *Pollia minor*, *Carex Koidzumii*, *Saussurea yamatensis*, *Cirsium kurobense*, *Salvia Mayebarae*.

309. Studies on the Hepaticae of Japan. IV. Yoshiwo HORIKAWA. (Jour. Sc., Hiroshima Univ. Ser. B, Div. 2, **1**, 1931, 13–34, 2 pls. and 10 text-figs.).

The following species are described in detail with figures: *Ptilidium Bisseti*, *Scapania gigantea*, *Leptocolea minutilobula*, *L. Nakaii*, *L. aoshimensis*, *Physocolea falcata*, *Lejeunea boninensis*, *Pycnolejeunea boninensis*, *Microlejeunea lunulatiloba*, *Lopholejeunea brunnea*, *L. Toyoshimae*, *Archilejeunea bidentata*. All are new, except the first species.

310. A Method of Sectioning Woody Structures. (Japanese). Gorô IDA and Kyôiti SATAKE. (Jour. Dendrol. Soc. **13**, 1931, 157–159, 2 text-figs.).

Hard and brittle woody structures are cut into 1 cm cubes, and according to WILLIAMSON they are boiled in distilled water, dipped into pure acetone for 1–2 hrs.,

and then placed in 12% acetone solution of cellulose acetate for a certain lapse of time, varying from 2 days to 2 weeks. The material is inserted between two celluloid plates which were previously treated by acetone for 1 minute. The whole is dried under diffuse light, and becomes easily sectionable after a certain time, 3–20 hrs. according to the kind of material taken.

311. A New Leaf-Blight Disease of the Grape-Vine. (Japanese with English résumé). Suehiko IKATA and Tsuyoshi HITOMI. (Ann. Phytopathol. Soc. Japan **2**, 1931, 357–373, 1 pl.).

A leaf-blight disease discovered in the Prefecture of Okayama some years ago is caused by a new fungus, *Acrosporum viticola*. It consists of conidial and ascigerous generation; the former is seen on the under surface of living leaves, while the latter is seen on the upper surface of fallen dead leaves. Inoculation has proven that it may infect *Vitis vinifera*, but not *V. Labrusca*. It grows best at 20–25°C, and the cytological study shows that it penetrates leaves through stomata. The diagnosis of the fungus is given.

312. On the Fungus-inhabiting *Cordyceps* and *Elaphomyces* in Japan. (With Japanese résumé). Sanshi IMAI. Trans. Sapporo Nat. Hist. Soc. **11**, 1929, 31–37).

3 species of *Cordyceps* and 3 of *Elaphomyces* are enumerated. The following are new and described: *C. Umemuraï* and *yezoensis*, *E. Miyabeanus* and *nopporensis*.

313. On tbe Clavariaceae of Japan. Sanshi IMAI. (Trans. Sapporo Nat. Hist. Soc. **11**, 1929, 38–44).

9 species of *Clavaria*, 2 of *Typhula* and 1 of *Pterula* are enumerated. The following are new and described: *C. quercicola*, *C. Shimadai*, and *T. Itoana*.

314. On the Clavariaceae of Japan. II. (With Japanese résumé). Sanshi IMAI. (Trans. Sappro Nat. Hist. Soc. **11**, 1930, 70–77).

7 species of *Clavaria*, 4 of *Typhula*, and 1 of *Pistillaria* are given. The following new are described: *C. lanceolata*, *C. meakanensis*, *C. Miyabeana*, *C. Tochinaiana*, *C. sachalinensis*, *T. alba*, *T. ishikarienses*, *T. subsclerotioides*, *Pistillaria Petasitidis*.

315. Heredity of Morning Glory. (Japanese). Yoshitaka IMAI. (Japan. Jour. Gen. **6**, 1930, 129–131).

In Morning Glory 111 factors were studied in detail till now, in which 7 groups of multiple allelomorphs and 12 mutable genes are included. 10 linkage groups are known. Three kinds of plastid mutations were discovered, of which the inheritance is maternal.

Bud-mutations are of two kinds, 1. those which originate in the embryo and 2. those which take place after the formation of the latter.

316. Karyological Studies on Some Plants of the Genus *Lycoris*. (Japanese with English résumé). Sukeo INARIYAMA. (Bot. Mag. Tôkyô **45**, 1931, 11–24, 3 pls. and 10 text-figs.).

The author made the comparative karyological studies on root-tip cells of 5 species of *Lycoris*, viz. *sanguinea*, *aurea*, *albiflora*, *radiata*, and *squamigera*. *L. sanguinea* has two sets of 11 rod-shaped chromosomes (diploid), and behaves quite regularly ; it is perfectly fertile. *L. radiata* is triploid, having three sets of 11 rod-shaped chromosomes ; the mitotic division is irregular and it is quite sterile. *L. squamigera* has besides 21 rod-shaped chromosomes 6 V-shaped ones, each of which is constricted in its middle portion and may be considered as two rod-shaped ones joined end to end ; its chromosome number is consequently $21+2\times6=33$, so that it is triploid, and is quite sterile. *L. aurea* has 2 rod-shaped and 10 V-shaped chromosomes, so that its chromosome number is $2+2\times10=22$. *L. albiflora* has generally 10 rod-shaped and 6 V-shaped chromosomes, so that its chromosome number is $10+2\times6=22$. *L. aurea* and *albiflora* are therefore diploid, and yet sterile. The reason of their sterility is not yet quite clear but it may be due to the presence of V-shaped chromosomes which are never present in *L. sanguinea* which is quite fertile. (Cf. No. 372).

317. On the Connection between the Nucleolus and the Spireme in *Hordeum*. (Japanese with English résumé). Choyo INOUYE. (Proc. Crop Sc. Soc. Japan **3**, 1931, 117-126, 1 pl.).

In the pollen mother-cells of *Hordeum sativum* undergoing the reduction division the author could discern the connection of the nucleolus with the spireme, not only in the prophase, but also in the interkinesis and even in the second telophase. Further, in the two latter cases the nucleolus and the spireme are connected at several points, while in the former only one point of connection is visible.

In the early strepsitene stage the spireme which is connected with the nucleolus contains much chromatin, and consequently seems dark. The latter fact indicates according to the author the transmission of chromatin from the nucleolus to the spireme to produce the chromosomes. The same phenomenon seems to take place also during the second prophase.

318. The Ascigerous Forms of Some Graminicolous Species of *Helminthosporium* in Japan. Seiya ITO and Kazue KURIBAYASHI. (Jour. Fac. Agric., Hokkaido Imp. Univ. **29**, 1931, 85-125, 4 pls.).

The ascigerous forms of *Ophiobolus Miyabeana* (=*Helminthosporium Oryzae*), *O. sativus* (*H. sativum*), *O. Setariae* (=*H. Setariae*) and *O. heterostrobus* (*H. Maydis*) were obtained in a culture. Though the form of conidia differs widely in the respective species, their perithecia are very similar.

The ascigerous form of *Pyrenophora graminea* (= *H. graminea*), *O. teres* (=*H. teres*), *P. japonica* and *P. Avenae* (*H. Avenae*) were collected in nature. Where the perithecia of these forms are abundantly produced is noticed in respect to each.

319. On the Nature of the Growth-promoting Substance Excreted by the "Bakanae" Fungus. (With Japanese résumé). Seiya ITO and Shoichi SHIMADA. (Ann. Phytopathyl. Soc. Japan **2**, 1931, 322-338, 1 pl.).

It is well known that the rice seedlings infected by the "Bakanae" fungus makes an extraordinary length growth which is clearly due to the stimulating action of a certain substance excreted by it. The authors have made the filtrate of the

culture solution of the fungus, and experiments were done on it. In the solution of fungus filtrate varying from 0.1–10% the greatest growth in length takes place. The filtrate is thermostable, because though even it is boiled and evaporated to complete dryness still it preserves its stimulating property. It is completely adsorbed by animal black and it can diffuse through semipermeable membrane.

320. On the Daily Fluctuation of the Osmotiv Value in Plants. Tadao JIMBO. (Sc. Rpts. Tôhoku Imp. Univ. Ser. IV, **6**, 1931, 285–306, 4 text-figs.).

According to the results of the investigation concerning the daily fluctuation of the osmotic value of a number of plants, as caused by the action of climatic conditions the author could find no such connection in their majority. Among a few which in this respect show positive behaviour *Polygonum Sachalinense* may be cited, where the fluctuation was most conspicuous on fine days immediately after rainy weather. Daily climatic changes have no conspicuous effect upon the magnitude of the osmotic value.

321. Chromosome Studies of a Species Cross in *Aegilops*. Fuyuwo KAGAWA. (Bull. Utsunomiya Agric. Coll. No. **1**, 1931, 57–60, 6 figs.).

The cross *Aegilops cylindrica* (n = 14) × *A. triuncialis* (n = 14) has given rise to F_1 plants which possess spikes of intermediate character (n=28). Bivalents, trivalents and univalents were seen in the meiosis of pollen mother-cells. It is probable that in the formation of bivalents in F_1 the allosyndesis takes place between some chromosomes of the two parents. Two F_1 plants have produced 7 kernels in all, being perhaps due either to self-fertilization or back-cross by *A. triuncialis*. They have given rise to 3 plants, of which the spikes are intermediate between the two parents, but not altogether similar to each other. In their pollen mother-cells the chromosome number was either 13 II + 3 I or 12 II + 2 I.

322. A Provisional List of Fungi Collected in Southern Saghalien. (With Japanese résumé). Katsumi KAWAI and Hiranao ÔTANI. (Trans. Sapporo Nat. Hist. Soc. **11**, 1931, 227–242, 4 figs.).

An enumeration of 108 species of fungi belonging to the Phyco-, Basidio-, and Ascomycetes. The following new species are described: *Puccinia Polygoni-Weyrichii*, *P. Sonchi-arvensis*, *P. Tokunagai*, and *P. tossoensis*.

323. Genomanalyse bei *Triticum* und *Aegilops*. II. *Aegilotricum* und *Aegilops cylindrica*. Hitoshi KIHARA. (Cytologia **2**, 1931, 106–156, 3 Taf. u. 34 Textabb.).

Die Arbeit zerfällt in zwei Teile. Im ersten, genetisch-karyologischen Teil werden die in der Einleitung zur I. Mitt. (1930) beschriebenen genomanalytischen Methoden bei der Untersuchung von TSCHERMAK's *Aegilotricum* (*Ae. ovata* × *T. durum*, fertil) und von *Aegilops cylindrica* zur Anwendung gebracht. Die wichtigsten Ergebnisse sind folgende:

1. Durch Kreuzungen von *Aegilotricum* mit *Ae. ovata* einerseits und *T. dicoccoides* (als Vertreter der Emmergruppe) andererseits wird einwandfrei bewiesen, dass die Genomgarnitur dieser künstlich hergestellten oktoploiden Pflanze tatsächlich aus

den beiden Emmer- und den beiden *Ovata*-Genomen besteht. Ihre somatische Genomformel ist danach (mit KIHARA's Genomsymbolen ausgedrückt): AA BB CC EE. Die dritte, mit *T. spelta* ausgeführte Kreuzung ergibt das auf Grund dieser Genomkombination erwartete Resultat und zeigt nebenbei, dass *Ae. ovata* kein Dinkelgenom enthält.

2. Zur Klarlegung der genomatischen Zusammensetzung von *Ae. cylindrica* führt Verf. Kreuzungen mit *T. durum* und *vulgare* aus. Auf Grund der in diesen Verbindungen beobachteten Chromosomenbindungen ist die Homologie eines der beiden *Cylindrica*-Genome mit dem Dinkelgenom D vollkommen sichergestellt (das andere *Cylindrica*-Genom ist mit einem der *Ovata*-Genome homolog).

3. Von intergenomatischen Bindungen werden die bei den früher untersuchten Bastarden (I. Mitt.) beobachteten bestätigt. Ausserdem berichtet Verf. über neue solche Bindungen zwischen den in Frage stehenden Weizen- und *Aegilops*-Genomen. Es sei hinzugefügt, dass Verf. die intergenomatische, lockere Chromosomenpaarung auf das Vorhandensein von homologen Partien in den betreffenden Chromosomen zurückführt (vgl. I. Mitt.).

4. Durch eine relative Anhäufung von Univalenten wird das Paarungsvermögen (in erster Reihe das intergenomatische) der sonst miteinander konjugierenden Chromosomen heruntergesetzt.

Auf den genetisch-karyologischen folgt der karyokinetische Teil, der sich in der Hauptsache mit dem Manövrieren der Univalenten befasst. Verf. beschreibt eingehend ihre Bewegungen während der I. Reifungsteilung und bringt eine eingehende Seriierung der metaphasischen Einzelstadien. Hauptergebnisse:

1. Die Univalenten der Bastardverbindungen im Weizen-*Aegilops*-Kreise zeigen im Verlauf der I. Metaphase in der Regel eine mehr oder weniger deutliche Tendenz sowohl zur Plattenbildung wie auch zur aequationellen Teilung. Die verschiedenen Intensitäten, mit denen diese beiden Tendenzen zum Ausdruck kommen, benutzt Verf. zu einer Einteilung aller von ihm bis jetzt untersuchten Bastarde inbezug auf das Univalentenverhalten.

2. Verf. ist auf Grund seiner umfangreichen Bastarduntersuchungen zur Ansicht gekommen, dass auch die Bewegungen der Univalenten bestimmte Gesetzmässigkeiten aufweisen. Nur dass uns hier eine viel grössere Labilität und Abhängigkeit von den äusseren Bedingungen, unter denen die Reifungsteilungen ablaufen, als bei den Bivalenten begegnet, so dass eine scheinbare Regellosigkeit zutage tritt. Diese Gesetzmässigkeiten fasst Verf. in klarer und übersichtlicher Weise im Rahmen einer mehrere Phasen enthaltenden "Norm" zusammen; die Phasen der Univalentenentwicklung sind hier im zeitlichen Zusammenhang mit denjenigen der Bivalentenentwicklung dargestellt. Verf.'s "Norm" ist eine künstlich konstruierte und besteht eigentlich aus einer Reihe von Postulaten. Nur die Tendenz zur Einhaltung der "Norm" ist bei jeder Verbindung vorhanden; häufige und mannigfaltige Modifikationen kommen in verschiedenem Grade überall vor, so dass das typische Verhalten einer bestimmten Bastardverbindung nur auf Grund eingehender statistischer Untersuchungen sicher festgestellt werden kann. Es können aber andererseits die Univalenten bei jeder Verbindung in den metaphasischen Einzelstadien ein mit der "Norm" übereinstimmendes Bild bieten, wenn das betreffende Stadium unter für

die Univalentenentwicklung "günstigen" Bedingungen abläuft. Im einzelnen ist von besonderem Interesse, dass die Univalenten im Weizen-*Aegilops*-Kreise eine mehr oder weniger ausgeprägte (bei manchen Bastarden eine sehr ausgesprochene) Neigung haben, sich in der früheren Metaphase in der Gegend der Pole zu zwei deutlichen Gruppen zu konzentrieren. Auch das sonderbare Einzelmanöver in den Bewegungen der Univalenten bei *Ae. cylindrica* × *T. vulgare*, in dessen Verlauf sie sich im Uebergangsstadium alle gleichzeitig normal zur Aequatorialebene orientieren und auf diese Weise ein Bündel um die Bivalentenplatte herum bilden, ist bemerkenswert.

3. Die Konfiguration der metaphasischen Univalentenplatte ist durch den Stemmkörper bestimmt. Sind Bindungen vorhanden, dann ist die Univalentenplatte immer ringförmig, weil der Stemmkörper dem weiteren Eindringen der Univalenten, auch nach dem Abwandern der Tochterchromosomen der Gemini, Widerstand leistet. Verf. konnte beobachten, dass die Grösse des freien Raumes inmitten der ringförmigen Univalentenplatte in direktem Verhältnis zum Umfang der Chromosomenpaarung, m. a. W. zur Grösse des Stemmkörpers, steht. Findet keine Paarung statt, dann bilden die Univalenten eine regelmässig ausgefüllte metaphasische Platte.

4. Verf. beschreibt die mit dem Vorhandensein von Univalenten in engem Zusammenhang stehende Erscheinung der Regression bei dem Bastard *Ae. cylindrica* × *T. vulgare*. Im Anschluss daran folgt eine Kritik der Bilder, die ROSENBERG als Belege für das Stattfinden der semiheterotypischen Teilung bei *Euhieracium*arten gegeben hat. Verf.'s Gedankengänge seien hier in aller Kürze wiedergegeben:

Allem Anschein nach besteht bei den Univalenten der von ROSENBERG untersuchten *Hieracium* eine deutliche Tendenz zur Plattenbildung. In diesem Falle dürfte ihre anaphasische Bewegung, die auf die Ansammlung im Aequator folgt, nicht vor dem Zutagetreten des Aequationsspalts einsetzen, was nach ROSENBERG gerade der Fall ist. Nach R. treten in der Anaphase die zufallsmässig verteilten Univalenten zu anaphasischen Tochterplatten zusammen, *ohne vorherige Längsspaltung*. Diese von R. beobachtete und mit Abbildungen belegte Erscheinung möchte Verf. dahin ausdeuten, dass es sich hier um keine Anaphase, sondern um die frühere Metaphase mit Konzentrierung der Univalenten an den Polen (die bei *Hieracium* auch vorzukommen scheint) handelt. Falls in Wirklichkeit unmittelbar darauf Interkinese folgt, wie R. annimmt, müsste es sich hier nach Verf. um einen Einzelfall der Regression handeln, die in den gegebenen Fällen in der früheren Metaphase einsetzt. Nach Verf. wäre also die semiheterotypische Teilung von R. ein Begriff, für den er keine adäquaten Tatsachen bei *Hieracium* gefunden bzw. abgebildet hat. Nach KIHARA bedeutet semiheterotypische Teilung: Zufallsmässige Verteilung der Univalenten in der Anaphase der I. Teilung *nach dem Zutagetreten des Aequationsspalts*, (bei Bastarden, deren Univalente eine Tendenz zur Plattenbildung haben), da ihre anaphasische Bewegungsfähigkeit erst mit dieser Erscheinung ausgelöst werden kann. Semiheterotypische Teilung in diesem Sinne ist aber nach Verf. bei Bastarden mit einer mehr oder weniger ausgesprochenen Tendenz der Univalenten zur Plattenbildung nur als Ausnahmefall zu erwarten. Einen solchen, bei den Weizen-*Aegilops*-Bastarden sehr seltenen Fall hat er bei *T. spelta* × *Ae. triuncialis* beobachtet und in Abb. 31–34 wiedergegeben.

Zum Schluss bespricht Verf. einige Möglichkeiten der Entstehung polyploider Reihen im Pflanzenreich.

Im Nachtrag diskutiert Verf. die Resultate einer Arbeit von MÜNTZING über *Galeopsis*-Bastarde, die ein schönes (und das erste!) Beispiel einer Genomanalyse an Hand von Kreuzungen zwischen passenden Analysatoren liefert, eine Methode, die Verf. in seiner I. Mitt. auf Grund theoretischer Erwägungen vorgeschlagen hat.

F.

324. Contributiones ad Salicologiam Japonicam IV. Arika KIMURA. (Sc. Rpts. Tôhoku Imp. Univ. Ser. IV, **6**, 1931, 185–197).

Die folgenden *Salix*arten und -varietäten usw. sind hervorgehoben oder beschrieben, mit Literatur und Synonymen: *S. Lackschewitziana*, var. *typica* nov. nom., var. *roridaeformis* nov. nom., *S. leptidostachys* (=*S. Miyabeanus*), *S. Nakamurana*, *S. Reinii*, var. *eriocarpa* var. nov., *S. yezoensis*, var. *angustifolia* var. nov., *S. kamikotica* sp. nov., *S. sendaica* sp. nov., var. *eriocarpa* var. nov.

325. Über eine neue *Aspergillus*art, *Asp. itaconicus* nov. spec. (Japanisch m. deutsch. Zfg.). Hirono KINOSHITA. (Bot. Mag. Tôkyô **45**, 1931, 45–61, 1 Taf.).

Aspergillus itaconus ist eine neue Pilzart, welche auf dem "Umesu", d.h. dem Saft der eingesalzten Pflaumen vorkommt und dort eine dünne Platte bildet. Sie wächst auf hochkonzentrierte Nährlösung und ist durch die reichliche Bildung von Itakonsäure in der Kulturlösung ausgezeichnet, wenn KNO_3 als N-Quelle benutzt wird. Sie bildet auch Mannit aus Rohrzucker, wenn die Nährlösung $NH_4 \cdot NO_3$ als N-Quelle enthält.

326. Studien über die Leuchtsymbiose in *Physiculus japonicus* HILGENDORF, mit der Beilage der zwei neuen Arten der Leuchtbakterien. Teijiro KISHITANI. (Sc. Rpts. Tôhoku Imp. Univ. IV. Ser., **5**, 1930, 801–823, 4 Taf. u. 3 Textabb.).

Im Muskelfleisch der Bauchwand von *Physiculus japonicus* befindet sich eine Drüse. In den Schläuchen derselben sieht man im Anfang eine Masse von Bakterien, welche nach ihrem Zerreissen auswärts gehen. Diese Bakterien, welche offenbar die Leuchtorgane des Fisches darstellen, sind neu und werden *Micrococcus Physiculus* genannt.

Aus der Hautoberfläche und dem Darmgang von *Physiculus* wurden leuchtende Wasservibrionen gezüchtet und *Microspira asamushiensis* n. sp. genannt.

Gegenüber dem Agglutinationsverfahren weisen die Stämme von *M. Physiculus* eine ausgesprochene Stammspezifität auf, während *M. asamushiensis* in dieser Hinsicht sich umgekehrt verhält. Auch die zwei obigen Bakterienarten verhalten sich agglutinatorisch voneinander ganz verschieden.

327. Report of the Biological Survey of Mutsu Bay. 18. Protozoan Fauna of Mutsu Bay. Subclass Dinoflagellata; Tribe Gymnodinioideae. Charles A. KOFOID. (Sc. Rpts. Tôhoku Imp. Univ. IV. Ser., **6**, 1931, 1–43, 3 pls.).

33 species are enumerated and described, of which 1 species of *Amphidinium*, 5 of *Gymnodinium*, 4 of *Gyrodinium*, 1 of *Cochlodinium*, 1 of *Nematodinium*, and 3 of *Pouchetia* are new.

328. On *Archeozostera* from the Izumi Sandstone. (Japanese). Kwan KORIBA and Shigeru MIKI. (Chikyû **15**, 1931, 165–204, 2 pls. and 10 figs.).

The Izumi Sandstone of Upper Cretaceous in Shikoku and Izumi contains, together with some marine animal fossils, a kind of plant impression, 2–8 dm in height, commonly called *Iris*-stone or fucoid. According to the authors it is a kind of sea grass closely related to *Phyllospadix* and *Zostera*, so it has been named *Archeozostera*.

The shoot, originally a rhipidate inflorescence, sprouting from rhizom distichously, has distichous leaves (spathe), each enveloping an axillary spadix, which often branches rhipidately, each accompanied by a spathe leaf. The mature fruits are arranged in one row, overlapping just as in *Phyllospadix*. By the shape, inclination and size of the spathe leaves, mode of branching, etc., 7 species have been determined so far. These 3 genera form a distinct systematic group—Zosteraceae, and should be included among Spadiciflorae in wide sense, but not to Helobiae.

Archeozostera adapted itself first to a shallow estuary, where it prospered luxuriantly with seasonal periodicity, tending then gradually to the marine life. The present state of distribution of Zosteraceae may be well explained, if one assumes the birth place of the family to be the bay of the Izumi Sandstone. S. MIKI.

329. Die Beziehungen zwischen den verschiedenen physiologischen Erscheinungen der Pflanzen und den verschiedenen Vegetationsorganen in Erscheinungen tretenden Farbstoffen. II. Mitteilung. Über die Beziehungen zwischen der Assimilationstätigkeit und der Anthocyanbildung bei *Abutilon Avicennae*. Hirosi KOSAKA. (Jour. Dept. Agric., Kyushu Imp. Univ. **3**, 1931, 29–45).

Für den Inhalt vgl. Japan. Jour. Bot. **5**, (65), Nr. 219.

330. Studies on the Absorption of Ammonia and Nitrate by the Root of *Zea Mays*-Seedlings, in Relation to the Concentration and the Actual Acidity of Culture Solution. Tsung-Lê LOO. (Jour. Fac. Agr., Hokkaido Imp. Univ., **30**, 1931, 1–118, with 1 plate and 26 text-figures).

By determining micro-analytically the amount of ammonia and nitrate absorbed by the root system of *Zea Mays* from culture solution containing ammonium nitrate, it was found that the unequal absorption of cation and anion from this salt is strongly affected by the concentration and the pH-values of the culture solution. When the concentration of the nutrient solutions is relatively high, ammonia is absorbed at first and nitrate is little or not at all absorbed by the seedlings of *Zea Mays*. Until ammonia is absorbed to a certain extent, the absorption of nitrate does not begin. On the other hand, if the concentration of the culture solution is low, nitrate is absorbed at very beginning of the experiment. The degree of reaction change caused by unequal absorption of ammonia and nitrate depends in the main upon the difference of amount of ammonia and nitrate absorbed. If the concentration of culture solution is very high, a much larger amount of ammonia than nitrate is absorbed by the seedlings, the acidity of the solution increases rapidly and considerably so that the growth of the seedlings is remarkably hindered. In the serious cases, the seedlings died from the extreme acidity. If the concentration of culture solution is low, nitrate is

absorbed as well as ammonia, therefore the increase of acidity is not so rapid and great as in the case of concentrated solutions.

If the concentration of ammonium nitrate is remarkably less than other salts in the solution, nitrate is better absorbed than ammonia. In this case, the change of reaction in the solution is not governed by the difference in absorption of ammonia and nitrate, the solution becomes more and more acid as the experiment proceeds.

When the seedlings are grown in the nutrient solution of different reactions the amount of ammonia absorbed increases with the decrease of acidity and increase of alkalinity of the culture solution. Nitrate, however, is relatively better absorbed at a weakly acid reaction. The absorption curve is wave-formed having more than three points of depressions. These points are respectively situated on the curve at pH 5.1–5.4 (usually at pH 5.2), pH 6.1–6.4 (usually at pH 6.4), pH 70–7.2 and sometimes at pH 7.8–7.9. This phenomenon is found in the experiments with different kinds of buffer mixture and culture solutions having uniform amount of either cations or anions.

In theoretical consideration of this absorption curve, the author adopted the hypothesis of polyisoelectric points of protoplasm which has been advanced by SAKAMURA and LOO in 1925 and regarded the points of depression as the isoelectric points of the protoplasm of the root or the resultant of the isoelectric phenomena.

When the yield expressed in dry weight of seedling grown in nutrient solutions of various reactions which are renewed every 24 hours is plotted against pH, a wave-like growth curve is obtained with many maxima and minima. The positions of the maxima and minima in this curve are coincident with those of the absorption curve or at least lie in the vicinity of them. In the case of extremely alkaline solutions, however, the growth curve shows just the opposite relation to the absorption curve. The amount of ammonia absorbed increases with the increase of alkalinity in the solution, but the yield in dry weight is inversely proportional to the alkalinity. In the solutions of the same reaction, the absorption of nitrogen and growth of seedlings are different, according to the difference in concentration of salts used as buffer and in ionic conditions of the nutrient solutions. Author.

331. Further Studies on Some Putrefactive Phytopathogenic Bacteria by Agglutinin Absorption. (With Japanese résumé). Takashi MATSUMOTO. (Jour. Soc. Trop. Agric. **2**, 1930, 16–25).

No. 216, which is the causing organism of soft-rot disease of Petsai (*Brassica pekinensis*), No. 197 from *Zinnia elegans*, No. 201 from radish, No. 204 from tomato, No. 212 from *Cucumis Melo* are more or less closely related serologically. This paper contains the results of serological experiments for differentiating all these bacteria. The agglutinin absorption of anti-no. 197 serum by the organism 197 removes the specific agglutinin almost completely, so that no further reaction of agglutination occurs with No. 197. Either with No. 216 or No. 204, however, a distinct reaction still takes place, so it is concluded that these two organisms, though somewhat resembling No. 197 serologically, are not quite identical with it. In the same way it was further ascertained that Nos. 201 and 216, though closely allied, are not quite identical to each other. No. 197 and No. 207 are pretty far off from each other. Nos. 204 and No. 197

seem on the basis of the results of agglutinin absorption to belong to one and the same species.

332. Genic Analysis in *Avena*. A Monograph. Hajime MATSUURA. (Jour. Fac. Sc., Hokkaido Imp. Univ. **1**, 1931, 76–107).

A review of the literature on *Avena*-inheritance. The bibliography contains 79 titles. Author.

333. Experimental Studies on the Saltation in Fungi. (Preliminary Report). V. On the Relation of Cultural Characteristics and Saltation to Time. (Japanese with English résumé). Isamu MATSUURA, (Trans. Tottori Soc. Agric. Sc. **3**, 1931, 154–160, 2 pls.).

Continuation of the author's experimental studies on *Ophiobolus Miyabeanus*. (Cf. Japan. Jour. Bot. **5**, (68)–(69), Nos. 229–232). The cultural characteristics of the fungus vary with the age of the inoculum. When that grown for about eight months is employed almost reddish mycelium was produced, while that grown for five months produced almost white mycelium. Again the inoculum grown for about three months and that grown for only twelve days produced gray and black mycelium or almost black one respectively.

It was the same with the occurrence of saltation. The saltant and variant produced by eight months inoculum were red, and those from five months one were almost white. Again from the three months inoculum many white islands were produced as saltant and variant, while the twelve days one gave rise only to few white islands as such.

334. Notes on the Larger Fungi of the San'in District II. (With Japanese résumé). Isamu MATSUURA and Yoshihisa KANADA. (Trans. Tottori Soc. Agric. Sc. **3**, 1931, 107–124, 2 pls.).

An enumeration of 118 species of fungi, collected in the San'in district, of which five are new to Japan.

335. Chromosome Number of Cultivated Plants III. Toshitaro MORINAGA and Eiji FUKUSHIMA. (Bot. Mag. Tôkyô **45**, 1931, 140–145, with figs.).

The number of chromosomes (either haploid or diploid) of the following plants was determined: *Sagittaria* (1 sp.), *Oryza* (1 sp. with 26 races), *Scirpus* (1), *Monochoria* (1), *Allium* (4), *Cardiocrinum* (1), *Lilium* (2), *Crocus* (2), *Calanthe* (1), *Paeonia* (1), *Dentaria* (1), *Euonymus* (1), *Impatiens* (1), *Thea* (1), *Ternstroemia* (1), *Cucumis* (1), *Aster* (1), *Gynura* (1), *Ligularia* (1) and *Solidago* (1).

336. Flora of the Prefecture of Iwate. (Japanese). Saburô MURAI. Morioka, 1930, 118 pp.).

An enumeration of 1538 species of plants, beginning with the Compositae and ending with the Hymenophyllaceae, collected in the Prefecture of Iwate in Northern Japan.

337. Morphology of the "Neck" of the Panicle as Related to the Resistance against Blast Disease in Rice Varieties. (Japanese). Isaburo NAGAI and

Arata IMAMURA. (Ann. Agric. Exp. Sta., Gov.-Gen. Chosen (Corea) **5**, 1931, 289–304, 3 pls.).

The examination of the anatomical structure of the neck of the panicle in 76 varieties of rice has revealed the fact that the number of stomata in this part is very various, depending on that of rows of stomata and that of the latter in each row. It was ascertained that the greater the number of stomata in the neck, the weaker the power of resistance against the attack of blast disease. It was however further ascertained that though in the foreign rice varieties the number of stomata in the panicle neck is much greater than in Japanese ones, yet the resisting power of the former is much greater than in the latter. This fact shows that this resistance might not depend simply on the number of stomata. Abundant manure contributes to the increase of the number of stomata.

338. The Chromosome Number in Cultivated and Wild Angiosperms. Goichi NAKAJIMA. (Bot. Mag. Tôkyô **45**, 1931, 7–11, 27 figs.).

The author has counted the chromosome number in root-tip cells and pollen mother-cells of species belonging to the genera *Quamoclt*, *Ipomaea*, *Calomyction*, *Calystegia*, *Anemone*, *Festuca*, *Melothria*, *Passiflora*, *Primula*, *Sorghum*, *Bromus* and *Arrhenatherum*. Some of the results of his observation are not in accord with those of other investigators.

339. Studies on Septorioses of Plants. III. On *Septoria Callistephi* GLOYER, Pathogenic on the China Aster. Hisao NAKAMURA. (Mem. Coll. Agric., Kyoto Imp. Univ. No. **13**, 1931, 23–32, 1 pl.).

The leaf-blight of *Callistephus chinensis* attacks usually leaves, but occasionally leaf- and flower-stalks. It is due to the attack of *Septoria Callistephi*, as proven by the author's inoculation experiments by its spores. The fungus grows on both agar and liquid culture media, and best at 20–28°C. On SAITO's soy agar a colony or a sector of salmon-orange color was produced. This mycelium continued to retain its characteristic color in spite of repeated transplantation on new soy agar. The pigment is easily soluble in alcohols and ether.

340. Accessory Food Substance in Relation to Growth of *Bacterium hyacinthi*. (Japanese with English résumé). Kakugoro NAKATA. (Ann. Phytopathol. Soc. Japan **2**, 1931, 339–349, 7 text-figs.).

It is well known that the microbes, especially pathogenic ones, though they can well thrive in beef bouillon or any other natural culture medium, they can not generally in synthetic media, such as CZAPEK's or USCHINSKY's, unless a certain natural substance will not be added as an accessory food. The author was successful in the culture of *Bacterium hyacinthi* in the solution of CZAPEK or USCHINSKY by the addition of bodies of *Bacterium solanacearum* or oryzanin, especially of the former. The accessory substance contained in each is destroyed by heating or diminished in its nutritive capacity by filtration. It may correspond to vitamine D, cleavage from vitamine B as shown by FUNK and DUBLIN. Its amount for the growth of the organism is fixed, being 3% in beef bouillon when oryzanin is the source of this

substance. It should not be under this percentage in order to be effective, though it may exceed it considerably without any influence whatever.

341. Fatuoidhafer und Chromosomenzahl. (Japanisch). Ichizo NISHIYAMA. (Japan. Jour. Gen. **6**, 1930, 186–187).

Die Individuen, die in bezug auf den Fatuoidcharakter heterozygot sind, zeigen gewöhnlich die Aufspaltung, Fatuoidtyp : Heterotyp : Kulturtyp = 1 : 2 : 1. Der Verf. hat eine eigentümliche Hafersippe bekommen, von denen die Aufspaltung F : H : K = 230 : 376 : 25 war. Nun zeigt die karyologische Untersuchung, dass wir bei Kulturtyp 2n = 42, bei Heterotyp 2n=41, und bei Kulturtyp 2n=40 haben. Bei der Reduktionsteilung des Heterotyps (2n=41) sind zweierlei Gameten produziert, d.h. dieselben mit 20 und 21 Chromosomen, und zwar im Verhältnis 6,22 : 1. Ihre freie Kombination bei der Befruchtung gibt somit Ind. mit 40 Chrom.: Ind. mit 41 Chrom.: Ind. mit 42 Chrom.=ungefähr 39 : 12 : 1. Als die Individuen mit 40 Chromosomen in den ersten Entwicklungsstadien beträchtlich aussterben (= 59, 22%), hat man *40 : 41 : 42* = 230 : 376 : 25, was die oben angedeutete Spaltungszahl erklären mag.

342. Studien über den Einfluss der Aussenbedingungen auf das Aufblühen der Reispflanzen. II. Pollenkeimung und Pollenschlauchwachstum. Yakichi NOGUCHI. (Japan. Jour. Bot. **5**, 1931, 351–369, 7 Textabb.).

343. Some Characters of *Sesamum indicum*, L. and their Inheritance. (Japanese with English résumé). Sigeroku NOHARA. (Japan. Jour. Gen. **6**, 1931, 180–185).

Seeds of *Sesamum indicum* are white, black or brown. The two latter kinds of seeds are stiff on account of the anatomical constitution of seed-coats, while the white ones are tender. The F_1 hybrids between stiff- and tender-seeded plants have stiff seeds, and in F_2 we have 3 stiff : 1 tender. Colored seed-coat is dominant over non-colored one : the F_2 segregation is rather complex and not discussed.

The quantity of crude protein, crude oil substance, carbohydrates and ashes, etc. was determined. The inheritance of the quantity of these substances in F_1 is apparently maternal, but this is due to the fact that in analysis whole seeds were used, incl. seed-coat which is a part of the P-plant.

344. Aschenbilder wichtiger Koniferenrinden Japans mit Rücksicht auf Systematik. Kametaro OHARA. (Mem. Coll. Agric., Kyoto Imp. Univ. No. **14**, 1931, 1–70, 8 Tafeln, 6 Fig.).

Es wurden mit den Aschenbildern der Baumrinden von 41 Nadelhölzern, worunter 38 Arten in Japan einheimisch sind, Untersuchungen vorgenommen, um dadurch deren Erkennung zu erleichtern und gleichzeitig ihre Verwandtschaft zu ermitteln.

Die Koniferenrinden führen ausnahmslos monokline Kalkoxalatkristalle, und zwar Einzelkristalle oder Kristallsand, welche die Hauptbestandteile des Aschenbildes ausmachen. Es fehlt den Koniferenrinden an Drusen, während *Ginkgo biloba* ausschliesslich diese Kristalle enthält, desgleichen finden sich bei den ersteren weder Sphärite noch Raphiden.

Die Einzelkristalle kommen als Zellinhalt nur bei den Pinaceae vor und lassen sich in zwei Grundformen scheiden, namlich in monokline Rhomboeder und Styloiden. Styloiden finden sich bei den *Tsuga*- und *Pinus* (Diploxylon)- Arten während monokline Rhomboeder bei den übrigen Pinaceae-Gattungen vorkommen. Sie werden meist von Zwillingen begleitet. Monokline Rhomboeder haben öfters die Neigung, sich lang zu strecken. Die isodiametrischen Einzelkristalle mit sechseckigen Flächen und deren Zwillinge, welche bei den Dikotylen sehr häufig auftreten, findet man bei den Koniferenrinden nicht.

Kristallsand tritt entweder als Zellinhalt oder in den Zellwänden auf. In den Korkzellen des Periderms von Pinaceae-Gattungen kommt Kristallsand weit verbreitet vor. Der Kristallsand tritt gewöhnlich in der innersten Schicht des Korkgewebes auf. Die Gattungen *Picea, Pinus, Pseudotsuga* und *Larix* zeichnen sich durch diese Kriställchen in den Korkzellen aus.

Abgesehen von den Pinaceae, enthalten alle Koniferenfamilien Kristallsand in den Zellwänden, weshalb die Gewebestruktur im Aschenbild gut erhalten bleibt. Bei den Taxodiaceae, Cupressaceae und Podocarpaceae ist pulveriger Kristallsand der radialen Wand des Bastes, und zwar der Mittellamelle, eingelagert. Im Aschenbild von Quer- und Tangentialschnitten lassen sich daher zahlreiche parallele Streifungen erkennen, während das radiale Aschenbild daneben Querlamellierung zeigt. Diese Querlamellierung rührt von der Ablagerung des Kristallsandes zwischen den Siebplatten her, welche in regelmässigem Abstand auf der radialen Wand vorkommen. Öfters tritt im Aschenbild die horizontale Wand der Markstrahlzellen als treppenförmige Streifungen hervor, da diese Wand bei den Taxodiaceae und Cupressaceae reichlich Kristallsand enthält.

Die Kristallsandkörner der Taxaceae und Araucariaceae sind der primären Lamelle der Sklerenchymzellen eingelagert und zeigen rhomboedrische Formen von isodiametrischem Habitus. Bei den Taxaceae sind nur die Sklerenchymfasern, und bei den Araucariaceae daneben die Steinzellen die Lagerstätte der Kalkoxalatkristalle. Die Cephalotaxaceae zeichnen sich durch die eigentümlichen Formen und die charakteristische Verteilung der Kalkoxalatkristalle in den Zellwänden aus. Mehrere Kristalle vereinigen sich mit ihren Kanten zu plattenförmigen Aggregaten und sind hauptsächlich der tangentialen Wand des Weichbastes eingelagert.

Die Formen und der Habitus der Kristalle sind sowohl in der Mittel- als auch der Innenrinde einheitlich und für die Gattungen bzw. Untergattungen charakteristisch. Sie können weder durch die Formen und die Grösse der Kristallschläuche noch durch Aussenfaktoren beeinflusst werden.

Der Kristallsand in den Zellwänden entsteht, gleichgültig ob er sich in der Mittellamelle (Cupressaceae, Taxodiaceae u. Podocarpaceae) oder in der primären Lamelle befindet, im Beginn des Zellebens und wird später mit Celluloselamelle bedeckt, während die Ausbildung der Einzelkristalle im Zelllumen (Pinaceae) mit der Degeneration des Zellinhaltes und der Zellwand Hand in Hand geht. Es lässt sich daher ein grosser Unterschied in der Lebensweise zwischen den Pinaceae und den übrigen Koniferenfamilien erkennen, da sich diese beiden Ausbildungsweisen der Kristalle nie bei einer und derselben Pflanze finden. Da die Formen und die Verteilung der Kalkoxalatkristalle in den Koniferenrinden nicht nur phylogenetische Eigenschaften sind,

sondern auch im ganzen Verlauf des Pflanzenlebens meistenteils unverändert bleiben, können sie als konstante Merkmale für die Erkennung der Arten herangezogen werden.

Die Koniferenrinden als Ganzes zeichnen sich durch folgende Charakterzüge aus: (1) Das Fehlen von Drusen, Sphäriten und Raphiden. (2) Die Kristalle sind arm an Flächen oder ihre Ausbildungsformen unvollständig. (3) Die Markstrahlen enthalten keinen Einzelkristall. (4) Das Fehlen von Kristallkammerfasern in wahrem Sinne des Wortes. (5) Das Vorkommen von Kristallsand von Kalkoxalat in den Korkzellen von Pinaceae. (6) Das häufige Auftreten von Kristallsand in den Zellwänden.

Die Koniferen können durch die Verteilung und die Formen der Kalkoxalatkristalle in der Rinde nach dem System PILGERS eingeteilt werden. Insbesondere unterscheiden sich die Pinaceae sehr scharf von den übrigen Familien, während die Taxodiaceae, Cupressaceae und Podocarpaceae sehr schwer voneinander zu trennen sind. Die Taxaceae und Araucariaceae zeichnen sich nur durch Lokalisation der Kristalle in den Sklerenchymzellen aus. Die Cephaltaxaceae zeigen ein ganz eigentümliches Aschenbild, was mit dem System PILGERS in Einklang steht.

Die Pinaceae lassen sich nach der Verteilung der Kristalle im Periderm und deren Habitus in der Mittel- und Innenrinde, nämlich ob Styloiden oder monokline Rhomboeder sind, in Gattungen einteilen. Es ist auffallend, dass die artenreiche Gattung *Pinus* in Untergattungen *Haploxylon* und *Diploxylon* eingeteilt werden kann, je nachdem die Kristalle in der Mittel- und Innenrinde Rhomboeder oder Styloiden sind.

Im speziellen Teile wurden Unterscheidungsmerkmale der Aschenbilder der betreffenden Baumrinden, nämlich die Verteilung und die Formen der kristallinen Elemente, diagnostisch dargelegt. Es wurden daneben topographischanatomische Strukturen der Rindengewebe jeder Arten beschrieben.

Die Arbeit enthält am Ende einen Schlüssel, womit man die Bestimmung der Koniferen in den bzw. Hölzer erleichtern kann. Autor.

345. Symbolae ad Floram Asiae Orientalis. II. Jisaburo OHWI. (Bot. Mag. Tôkyô **45**, 1931, 183-197).

Among the plants enumerated by the author the following are new: *Aristida Takeoi, Elymus villosulus, Scirpus abactus, S. hondoensis, Eriophorum scaborium, Calamagrostis adpressus-ramea, Festuca extremorientalis, Poa deschampsioides, Eriocaulon sphagnicolum.*

346. Geschlecht bei *Stropharia semiglobata*. (Japanisch). Kôhei OIKAWA. (Bot. Mag., Tôkyô **45**, 1931, 250-257).

An *Stropharia semiglobata*, welche an einem gewissen Gegend in Sendai, und zwar auf Pferdmist wächst, hat der Verf. einige Experimente ausgeführt. Daraus hat er nämlich die Einspormyzelien bekommen, und dabei hat er zweierlei Gruppen unterschieden (+ oder −), welche entweder untereinander Schnallen bilden können oder nicht, d.h. die Getrenngeschlechtigkeit sowie die Bipolarität dieser Pilzmyzelien wurde experimentell nachgewiesen. Aus einem andern Gegend wurde die gleiche Pilzart gesammelt, und es wurde dabei festgestellt, dass die daraus hervorgegangenen Einspormyzelien ohne Ausnahme zusammen mit denselben aus dem oben gennanten

Pilze Schnallen bilden können. Diese zweierlei Pilze, welche morphologisch zueinander übereinstimmen, können somit geschlechtlich als verschiedene Standortsrassen betrachtet werden. Aus den Schnallen, welche durch die Vereinigung der Einzelmyzelien dieser Standortsrassen ausgebildet worden sind, sind die normalen Fruchtkörper entwickelt, und weiter an den letzteren wurde die Getrenntgeschlechtigkeit und Bipolarität der Einspormyzelien klar erkannt, ganz gleicherweise wie bei den Elternmyzelien.

347. Über die Polyploidie der Gattung *Senecio*. (Japanisch). Sakuichi OKABE. (Bot. Mag. Tôkyô **45**, 1931, 258–260, 8 Textabb.).

Der Verf. hat die haploide Chromosomenzahl bei einigen *Senecio*arten bestimmt, welche 10, 20, 24 oder 40 beträgt. *S. palmatus* (n = 20)×*S. cannabifolius* (n = 40) hat den Bastard mit 60 Chromosomen in seiner Soma gegeben. Dabei geht die meiotische Teilung der Pollenmutterzellen unregelmässig vor, und wenn die Pflanze selbst üppig wächst und blüht jedes Jahr, ist sie ganz steril.

348. Contribution to the Knowledge on the Soil Microflora of *Pseudosasa*-Association. I. Yônosuke OKADA. (Sc. Rpts. Tôhoku Imp. Univ. IV. Ser., **6**, 1931, 149–162, 3 text-figs.).

Pseudosasa kurilensis MAKINO is a bamboo widely distributed in mountainous regions of Japan. The author's laboratory in Mt. Hakkôda in Northern Japan stands amidst the association of this bamboo. He makes a physiological study of soil microflora in this region. A certain quantity of soil taken out from the depths of 3–6, 30, 50 cm was inoculated on a certain culture medium and the cellulose decomposition as well as the denitrification were studied both under aerobic and anaerobic condition. As to the former action the DUBOS' medium was employed, and it was observed that with soil from 3–6 cm depth and under aerobic condition the cellulose decomposition is due to the action of moulds, and not to that of bacteria. With soils from deeper layers no cellulose decomposition takes place at all. Under anaerobic condition however, both with soils from 3–6 and 30 cm depths the cellulose decomposition by bacteria was found to take place.

The denitrification is not very vigorous, unless a large quantity of soil will not be added, and then the complete destruction of N_2O_3 was proven in several cases. No increment of NH_3 was detected; on the contrary, its small decrement was seen. The conclusion is that the vigorous reduction of N_2O_5 to N_2O_3 was not to be expected and the production of NH_3 seems improbable.

The evolution of gas was noticed in some cases, so that the microbes which will reduce N_2O_3 to N gas may be present in the soil under experiment.

349. On the Marine Algae from Kôtôsho (Botel Tobago). Kintaro OKAMURA. (Bull. Biogeogr. Soc. Japan **2**, 1931, 95–122, 2 pls., 1 text-fig.).

An enumeration of marine algae collected in the small island Kôtôsho (Botel Tobago) at the southern extremity of Formosa. 29 Chloro-, 16 Phaeo-, and 47 Rhodophyceae are given, of which the following three are new and described: *Chamaedoris orientalis*, *Sargassum prolongatum*, and *Carpopeltis formosana*.

350. Beiträge zur Kenntnis der rosafarbigen Sprosspilze. Kazuo OKUNUKI. (Japan. Jour. Bot. **5**, 1931, 285–322, 1 Taf. und 22 Textfig.).

351. On *Fraxiniopsis* WIELAND and *Yabeiella* ÔISHI, gen. nov. Saburô ÔISHI. (Japan. Jour. Geol. and Geogr. **8**, 1931, 259–267, 1 pl.).

Fraxiniopsis is a genus erected by WIELAND for a fossil seed or fruit type obtained from the Rhaetic strata of Minas de Petroleo, southwest of Mendoza, Argentina. The fossils are alate objects, and WIELAND thinks them to be a Dicotyledon related to *Fraxinus*. It comprises two species, *F. major* and *minor*. The author has got an alate fossil from Argentina identical with WIELAND'S *F. minor*. What WIELAND thinks to be two cotyledons the author takes for two seeds arranged side by side, and since the general feature of ala is very similar to the lamina or sterile portion in *Cycadocarpianum*, he thinks that *Fraxiniopsis* is gymnospermous and may be an instance of Hemi-Conifer WIELAND'S. The diagnosis of *F. minor* is given.

Another fossil associated with *F. major* which was not named by WIELAND is a kind of foliage characterized by a peculiar type of nervation with marginal and lateral nerves, any two adjacent ones of which occasionally join near the margin of the leaf. The author created a new genus *Yabeiella* which contains six species.

352. A New Type of Fossil Cupular Organ from the Jidô Series of Korea. Saburo ÔISHI. (Japan. Jour. Geol. and Geogr. **8**, 1931, 353-356, with figs.).

A group of cupules was collected in black carbonaceous state in the third pit of the Jidô coal mine near Heizyô, Northern Korea. A new genus *Koraia* is created, containing one species *K. koraiensis*.

353. *Yabeiella* sp. from the Japanese Triassic. Saburô ÔISHI. (Japan. Jour. Geol. and Geogr. **8**, 1931, 357–359, with figs.).

A species of *Yabeiella* was found in Japan in the Upper Triassic formation of Nariwa, Province Bittyû.

354. Systematische und anatomische Studien über die japanischen Juncaceen (1). (Japanisch). Yosisuke SATAKE. (Bot. Mag. Tôkyô **45**, 1931, 235–249, 4 Abbildungsgruppen).

Eine Anzahl von japanischen *Juncus*arten können nach der anatomischen Struktur ihrer Karpellen unterschieden werden. Der Verf. gibt eine übersichtliche Schlüssel dieser Arten sowie ihre Beschreibung nach diesen Merkmalen an. Er unterscheidet vor allem nach BUCHENAU drei Typen, je nachdem der Fruchtknoten einzellig, unvollständig oder vollständig dreizellig ist. Die folgenden anatomischen Merkmale sind dann in Erwägung genommen: 1. ob das distale Ende der Scheidwände hutförmig angeschwollen ist oder nicht, 2. ob die äussere Oberhautzellen ganz herum oder bloss U-förmig (innen und seitlich) verdickt sind, 3. ob die innere Oberhautzelle verholzt ist oder nicht, 4. die Zahl der Parenchymzellschichten zwischen den inneren und äusseren Oberhaut und ob diese Parenchymzellen verholzt sind oder nicht, 5. ob die Oberhautzellen des hutförmigen Teils verdickt sind oder nicht und ob sie mit verdickten und verholzten Zellgruppen versehen sind oder nicht, 6. die Zahl der Gefässbündel im

Plazenta, 7. ob die Zellen der Dehiszenzteile verholzt sind oder nicht, und 8. die Dicke und Lange der Karpellen.

355. Studien über die Wirkungen der durch *Ophiobolus Miyabeanus* gebrauchten Lösungen auf die Keimung und Entwicklung eines anderen Pilzes. Seiichi SATOH. (Mem. Coll. Agric., Kyoto Imp. Univ. No. **13**, 1931, 41–54, m. 2 graphischen Darstellungen).

Ophiobolus Miyabeanus (= *Helminthosporium Oryzae*) wurde in den RICHARDschen Nährlösung kultiviert und nach gewisser Kulturdauer wurde der auf die Keimzahl und die Keimschlauchlänge gegründete Keimeffekt von *Aspergillus niger* in der gebrauchten Nährlösung gemessen. Danach ist die hemmende Wirkung der während 1-2 Wochen gebrauchten Lösung nicht merklich gross, aber in der 8 Wochen alten Lösung wird sie plötzlich stark, und in der 6 Wochen alten beginnt die Autolyse des Pilzkörpers einzutreten. Wenn aber man die gebrauchte Lösung durch das CHAMBERLANDschen Tonfilter filtriert, kann man gar keine hemmende Wirkung nachweisen, woraus man schliessen kann, dass die gebrauchte Lösung beide die das Wachstum hemmende und es beschleunigende Stoffe enthält und das Tonfilter die letzteren passieren lässt und die ersteren zurückhält. Die Wirkung der RICHARDschen Lösungen, von denen die Konzentration ihrer Bestandteile verschieden ist, wurde untersucht, mit keinen besonderen Einfluss auf den Keimeffekt. Es wurde weiter aufgefunden, dass die Lösungen, die eine grössere Pilzernte geben, eine grössere Hemmung ausübt, woraus man sehen kann, dass die Hemmung auf die Wirkung der Stoffwechselprodukte des Pilzes zurückzuführen ist. Wenn man die gebrauchten Lösungen mit Wasser verdünnt, geht die hemmende Wirkung leichter verloren als die fördernde. Weiter gegen Hitze ist die letztere Wirkung viel beständiger als die erstere, welche leicht durch Wärme zerstörbar ist.

356. Reports on Formosan Fungi. V. (Japanese). Kanekichi SAWADA. (Rpts. Res. Inst. Formosa, Agric. Dpt. **51**, 1931, 131 pp. text, 5 pls., 14 pp. index).

In this report the author enumerates 128 species of fungi belonging to the Archi-, Phyco-, Asco-, Basidiomycetes and Fungi Imperfecti found in various parts of Formosa. The following are new and described: *Phytophthora Cyperi-lriae, Plasmopara Chrysanthemi-coronarii, Meliola Acaciae-confusae, Zukaliopsis Gardiniae, Aleurina nigrodisca, Nectria citricola, N. Pterospermi, Melampsora Salicis-Warburgi, Phakopsora circumvallata, Triphragmium formosanum, Uredo Milletiae, Septobasidium Parlatoriae, Exobasidium Pieridis-ovalifoliae, Anellaria ochroleuca, A. planiscula, Phoba Lebbeck, Hendersonia citricarpa, Sphaeceloma Batatas, Gloesporium Hibisci-tiliacei, Cylindrosporium Phaelaenopsidis, Cladosporium sclerotiophilum, Helminthosporium Citri, Spondylocladium Tremae, Alternaria americana, Cercospora Lagerstroemiae-subcostatae, C. Panici-miliacei.*

357. Chromosomenzahlen und Phylogenie bei der Gattung *Potentilla*. Naomasa SHIMOTOMAI. (Jour. Sc., Hiroshima Univ. Ser. B, Div. 2, **1**, Art. 1, 1931, 1–11, 1 Textfigurengruppe).

Für den Inhalt dieses Aufsatzes vgl. Japan. Jour. Bot. **5**, (78), Nr. 262.

358. Bastardierungsversuche bei *Chrysanthemum*. I. Naomasa SHIMOTOMAI. (Jour. Sc., Hiroshima Univ. Ser., B, Div. 2, **1**, Art. 3, 1931, 37-54, 3 Taf., 14 Textabb.).

Der F_1-Bastard zwischen *Chrysanthemum Decaisneanum* (n=36) und *C. indicum* (n=18) nähert sich äusserlich mehr der ersteren Art als der letzteren, was auf die grössere Chromosomenzahl der ersteren zurückzuführen ist. Der Bastard zeigt 36+18=54 diploide Chromosomen. Bei der Reduktionsteilung der Pollenmutterzellen kann man 27 Gemini nachweisen, und alle Vorgänge dieser Teilung, beide hetero- und homoetypisch, gehen ganz regelmässig vor sich. Es ist klar, dass unter 27 Gemini wenigstens 9 durch Autosyndese der *Decaisneanum*-Chromosomen gebildet werden müssen. Bei dem F_1-Bastard, *C. marginatum* (n=45) und *morifolium* (n=27) sieht man fast gleiche Verhältnisse, inbezug sowohl auf das äussere Aussehen als auch die Chromosomenzahl, woraus man auch hier auf das Vorkommen der Autosyndese gewisser *marginatum*-Chromosomen schliessen kann.

Bei dem Bastard, *C. marginatum* (n=45) und *C. indicum* (n=18) ist die Zahl der somatischen Chromosomen 63. Bei der Reduktionsteilung der Pollenmutterzellen weist man 18 bivalente und 27 univalente nach. Jedes von den ersteren Chromosomen wird aus je einem *marginatum*- und einem *indicum*-Chromosom zusammengesetzt werden, während alle letzteren aus *marginatum* herrühren und unpaaring bleiben. (Vgl. Japan. Jour Bot. **5**, (78), Nr. 263).

359. Investigations on the Relation between Plants and Their Surrounding Conditions by the Quantitative Method. IV. On the Variation of Wavyness in the Lateral Walls of Epidermal Cells of Leaves in Sunshine and Shade Individuals in the Same Species. (Japanese with English résumé). Makoto TAKENOUCHI. (Bult. Sc. Fak. Terk., Kjusu Imp. Univ. **4**, 1931, 191-217).

In reference to epidermal cells of leaves the author had measured the degree of wavyness of their lateral walls in the individuals belonging to several species of herbs which are growing under sunshine and shade. The measurement is based on the formula $m=L:L'$, of which m denotes the degree of wavyness, L the actual length of wavy lateral wall in longitudinal section, and L' the circumference under the supposition that the section of the cell were a circle. To cite a few examples of the results of measurement: m = 1,67 (*Rumex acetosella*), 1,17 (*Ipomaea batatas*), 1,22 (*Oenothera odorata*), 1,3 (*Aucuba japonica*), 1,41 (*Stellaria media*), 1,66 (*Justicia procumbens*), etc. It was found that the deeper the shade, the greater m.

360. Systematisch-vergleichende Morphologie und Anatomie der Vegetationsorgane der japanischen Bambusarten. Yoshio TAKENOUCHI. (Mem. Fac. Sc. & Agric., Taihoku Imp. Univ. **3**, 1931, 1-60, 3 Taf. u. 29 Textfig.).

Indem die systematische Diagnosierung der Bambusarten nach ihren Blütenorgane nicht immer durchführbar ist wegen der grossen Seltenheit des Blühens, ist ihre Unterscheidung nach dem anatomischen Merkmale ihrer Vegetationsorgane wünschenswert, woher die vorliegenden ausführlichen anatomischen Studien Verfassers herrühren.

Wegen des fast ausschliesslich beschreibenden Charakters der vorliegenden Abhandlung ist es nicht möglich, eine gedrängte Übersicht des Inhaltes hier anzu-

geben. Unten sei kürzlich einige Ergebnisse des Verfs. Untersuchungen hervorgehoben.

1. Es gibt vier Typen der Verzweigungsmodi des Rhizoms, nämlich, einfache Rasenbildung, Rasenbildung aus Ausläufern, seitliche Rasenbildung aus Rhizomen, und zerstreute Verzweigung.

2. Der Halm besteht aus drei Teilen, nämlich, dem unterirdischen gestauchten Zweigbasis oder Stiel, Übergangshalm oder Stengelbasis und dem echten Stengel oder Halm.

3. Die äusserste kleinen Bastbündel der Internodiumbasis des Halmes sind in der Längsrichtung je nach der Art mehr oder minder nach aussen hin gekrümmt.

4. Die Vorblätter sind je nach der Spezies geschlossen oder gespalten.

5. Die Haare auf dem Vorblattflügelrand des horizontalen Rhizoms und des Stengels sind ein- oder mehrzellig.

6. Die Zahl der Knospen in demselben Vorblatt ist nach der Spezies und nach der Lage am Stengel verschieden: sie beträgt 1–7.

Ein Bestimmungsschlüssel verschiedener Gattungen nach den anatomischen Merkmale ist angegeben.

361. Morphologische und entwicklungsmechanische Untersuchungen bei japanischen Bambus-Arten. Yoshio TAKENOUCHI. (Mem. Coll. Sc., Kyoto Imp. Univ. Ser. B **6,** 1931, 109-160, 3 Taf. u. 43 Textfig.).

In der vorliegehden Abhandlung beschreibt der Verf. ausführlich eine grosse Anzahl von Einzelheiten, welche er bei seinen morphologischen und anatomischen Studien der japanischen Bambusarten beobachtet hat. Unten wird die Zusammenfassung der Ergebnisse seiner Studien in den eigenen Worten des Verfs. zitiert werden.

1. Bei *Phyllostachys reticulata* bildet sich das horizontale Rhizom, wenn es im Boden auf ein Hindernis stösst, spontan zum aufgerichteten Halme um, dessen Basis gewöhnlich mit einem soliden Mark versehen ist.

2. In Bezug auf die Entwicklung des Bambus-Schösslings lassen sich folgende zwei Typen unterscheiden, 1. sukzedane Ausbildung (*Dendrocalamus, Bambusa*), 2. simultane Ausbildung (*Phyllostachys*).

3. Bei *Phyllostachys*arten wird jedes Halminternodium von auffälligen Rinnenstreifen durchzogen, während bei anderen Gattungen diese nur schwach entwickelt sind. Diese Rinnen sind um so stärker, je grösser die Knospen sind, die auf die simultan gebildeten Halminternodien einen Druck ausüben.

4. Bei *Phyllostachys reticulata* var. *Marliacea* zeichnet sich die Halmoberfläche durch viele gerade laufende Furchen aus. Die Verkleinerung und Vereinigung der Gefässbündel scheinen an dieser Furchenbildung beteiligt zu sein.

5. Bei *Phyllostachys* gibt es gabelig verzweigte sowie verbänderte Halme.

6. An dem Bambushalm lassen sich nach folgenden anatomischen Merkmalen drei Teile (echter Halm, angeschwollene Halmbasis und Stiel) leicht unterscheiden, 1. Gefässbündelstruktur. 2. Dickenverhältnisse der Rindenschicht, 3. Epidermiszellen und ihre Verteilung.

7. Die frühzeitig sich aus dem Gewebeverband lösenden Markzellen, welche später die Halminnenfläche bedecken lassen sich nach ihrer Entstehung in folgenden drei Typen einteilen, 1. dünnes, papierartiges Markhäutchen (*Phyllostachys*arten), 2. fleckenweise auftretende, zerrissene Markzellmassen (*Pleioblastus*, *Yadakeya* u.a.), 3. pulver- oder körnerähnlicher Überzug (*Bambusa* und *Dendrocalamus*).

8. Die Form der Grundparenchymzellen des Halmes schwankt zwischen Lang- und Kurzzellen, neben Übergangszellen. Die Anzahl dieser letzteren ist verhältnismässig kleiner als die der beiden ersteren, und dadurch kommt eine Kurve mit zwei Gipfeln zustande (mit Ausnahme von *Arundinaria niitakayamensis*), wenn man die Länge der Zellen graphisch darstellt.

9. Bei *Chimonobambusa quadrangularis* sowie in den soliden Halmen von *Phyllostachys reticulata* wird die regelmässige Gefässbündelanordnung im Querschnitt des Halmes oft gestört, und die Orientierung des Gefässbündels ist gedreht.

10. Bei *Phyllostachys edulis* und *P. reticulata* sind die Diaphragmen gewöhnlich nach unten gekrümmt, nur selten nach oben. Die erstere Krümmung wird durch konzentrische Differenzierung der Diaphragmen um den Vegetationspunkt herum verursacht, während die letztere durch frühzeitigen Schwund des Markgewebes stellenweise entsteht.

11. Die Lochbildung des Diaphragms von *Phyllostachys edulis* beruht darauf, dass die dicht übereinanderliegenden Diaphragmen im Jugendstadium des Schösslings miteinander verwachsen; durch nachträgliche Trennung im Laufe der Halmentwicklung kommt ein Loch zustande.

12. Bei *Phyllostachys reticulata* var. *aurea* is der Halm unmittelbar unterhalb des Knotens zu einem ringförmigen, fleischigen Teile ausgewölbt. Dies kommt dadurch zustande, dass die Halmwand in ihrem Entwicklungsstadium durch die seitlich erzeugte Ausbuchtung der Brachysklereiden eine Krümmung nach auswärts bekommt, wodurch die fleischige Wölbung gebildet wird.

13. Bei *Phyllostachys reticulata* habe ich einmal eine einseitige Wucherung des Halminternodiums gesehen, welche durch einseitige Knotenbildung verursacht war.

14. Bei *Phyllostachys reticulata* var. *aurea* erfahren einige untere Internodien manchmal ein anomales Wachstum, infolgedessen der Halm verkürzte Internodien bekommt. Ihre Zellen sind auch merklich verkürzt.

15. Bei *Phyllostachys edulis* var. *heterocycla*, *P. formosana* u.a. erfahren einige untere Internodien manchmal ein anomales Wachstum, indem die periodische Knotendifferenzierung in der Halmbasis eine starke Störung erfährt; einige aufeinanderfolgende Diaphragmen kommen nahe aneinander zu liegen oder verwachsen seitwärts miteinander und infolgesdessen entsteht der zickzackförmige Verlauf der Halmknoten.

16. Bei *Pleioblastus*, *Yadakeya* u.a. sind die verkümmerten Blattspreiten des Schösslings aufrecht und glatt, während die der *Phyllostachys*arten runzlig sind. Die Verrunzelung kommt dadurch zustande, dass die verkümmerten Blattspreiten im Jugendstadium von den schmalen Lücke übrig lassenden, nahezu simultan erzeugten Scheiden umschlossen sind; infolgedessen wird die Entwicklung einer glatten Oberfläche gehemmt. Geht die Entwicklung der Halminternodien sukzedan vor sich, so

treten die Scheiden und die verkümmerten Blattspreiten allmählich heraus, sodass die Spreite sich glatt strecken kann.

17. Bei *Phyllostachys, Shibataea* u.a. fallen die Scheidenblätter in akropetaler Reihenfolge schon vor der völligen Entwicklung des Schösslings ab, während sie bei *Pleioblastus, Yadakeya* u.a. ohne Abstossung zugrunde gehen und allmählich am Orte ihrer Enstehung zerfallen. Dieser Unterschied kommt dadurch zustande, dass die Trennungsschicht der Scheidenbasis, welche bei den zuerst genannten Arten stets zu sehen ist, bei den letzteren gänzlich fehlt.

18. Bei *Phyllostachys reticulata* var. *aurea* besteht ein Zweigsystem aus ca. 6–10 Blättern. Jeder Zweig wirft seine Blätter nach und nach von unten nach oben ab, und schliesslich nach zwei Jahren gehen alle Blätter zugrunde. Der Tod des Hauptzweigs und der Zweiglein folgt auf die Ausbildung der scheinbar endständigen Blattspreite, die zwei Jahre nach der Knospenaustreibung fertig entwickelt ist.

19. In Bezug auf den Verfärbungsprozess des Blattes lassen sich folgende zwei Typen unterscheiden, 1. Randverfärbung (*Sasa, Dendrocalamus*), 2. basipetale Verfärbung (*Phyllostachys, Shibataea*) u.a.

Anatomisch sind die Blattspreite von diesen beiden Typen in folgender Weise verschieden, 1. bei dem ersteren sind die Gefässbündel weit voneinander entfernt, mit grösseren Luftgängen dazwischen, 2. bei dem letzteren sind die Gefässbündel dicht zusammengerückt, mit sehr kleinen oder keinen Luftgängen dazwischen.

20. Bei *Yadakeya japonica, Y. Otawarii, Pseudosasa spiculosa, Semiarundinaria fastuosa* u.a. treten die Knospen am unteren Teile des Halmes nicht hervor. Man sieht nur ein hellgrünes Pünktchen an der betreffenden Stelle. Man findet hier innerhalb der Rinde eine Knospenanlage, welche unentwickelt bleibt.

21. Die Zahl der Verzweigungen des Hauptstammes ist je nach den Gattungen sowie de Lage am Halme verschieden. Am mittleren Teile des Halmes ist sie wie folgt, 1. *Sasa, Sasaella, Yadakeya, Pseudosasa* 1, 2. *Phyllostachys* 2, 3. *Pleioblastus, Semiarundinaria, Sinobambusa, Chimonobambusa* u.a. 3, 4. *Shibataea* 5, 5. *Dendrocalamus* und *Bambusa* 7–9.

362. A Soft Rot of Sugar-beet and its Causal Organism. (Japanese with English résumé). Seito TAKIMOTO. (Ann. Phytopathol. Soc. Japan **2**, 1931, 350–356).

A disease of sugar-beet prevailing in Northern Korea, causing yellowish soft rot and peculiar odour, is due to a new organism, *Bacillus betivorus*. The diagnosis is given. Inoculation experiments show that it can infect roots of carrot and radish, tubers of potato and fruit of tomato.

363. Studien über die Ernährung der höheren Pflanzen mit den organischen Verbindungen. Isuke TANAKA. (Japan. Jour. Bot. **5**, 1931, 323–350, 3 Textfig.).

364. Studies on a New Canker Disease of Japanese Pear Trees Caused by *Phomopsis Fukushii* n. sp. Shoichi TANAKA and Shigeru ENDO. (Trans. Tottori Soc. Agric. Sc. **2**, 1931, 123–134, 2 pls.).

The diagnosis of a new species, *Phomopsis Fukushii* is given. It affects Japanese pear-trees: it invades cut ends of branches as well as wounded trunks and limbs, and

causes dark brown canker on them The degree of resistance against this disease is somewhat different in different pear varieties. Apple trees are quite immune. For the mycelial growth of this fungus ±8°C is the minimum, and ±33°C the maximum.

365. Contribution to the Knowledge of *Citrus* Classification Nos. 1-2. (Japanese with English résumé). Tyôzaburô TANAKA. (Studia Citrologia **3**, 1929, 164–188).

The term citrology involves many branches of science relating to *Citrus* and its relatives. The author discusses in No. 1 of the paper several methods necessary for pursuing taxonomic citrology. In No. 2 he points out five native ranges of *Citrus* fruits, viz. Chinese, Indian, Japanese, Malayan and East Indian regions, and gives a tentative list of *Citrus* species compiled from his unpublished monograph.

366. On the Origin of *Citrus* Species. (Japanese with English résumé). Tyôzaburô TANAKA. (Studia Citrologia **4**, 1930, 1–22).

The genus *Citrus* is classified by the author as follows:

Subgenus Archicitrus containing 5 sections, Papeda, Limonellus, Citrophorum, Cephalocitrus, Aurantium;

Subgenus Metacitrus containing 2 sections, Osmocitrus and Acrumen. The latter is divided into 3 subsections, Euacrumen, Microacrumen and Pseudofortunella.

The facts which justify the above classification are given.

367. On the Centre of the Origin of the *Citrus* Fruits. (Japanese with English résumé). Tyôzaburô TANAKA. (Studia Citrologica **4**, 1931, 179–205).

The native home of all important species of Archicitrus is East India, where also later developed Metacitrus occurs abundantly. Hence the author considers East India as the centre of the origin of *Citrus* fruits. The South China coastal region considered so far as the most important native home of *Citrus* fruits is according to the author's view but an extension of Indian region.

368. Epochs in the History of Pre-Linnean Botany. (Japanese with English résumé). Tyôzaburô TANAKA. (Trans. Nat. Hist. Soc. Formosa **22**, 1931, 99–112).

According to the author six epochs of botanical studies may be distinguished before the appearance of LINNAEUS' Species Plantarum in 1753. He enumerates the chief epoch-makers and discusses their respective achievement concerning the enumeration of plants, phytography and phyto-iconography, plant organography and phytotomy, nomenclature, and phyto-taxonomy. Valerius CORDUS, Conrad GESNER, Aluigi ANGUILLARA, Ulyssus ALDROVANDI are mentionen as most prominent among others.

369. Studies on the Appearance of Mutations in the Morning Glory. (Japanese). Hirosi TERAO and Nagaharu U. (Japan. Jour. Gen. **6**, 1931, 195–198, 1 fig.).

During the culture experiments which have extended from 1917 to 1921 the authors have seen the appearance of 11 gene-mutations, of which except two the mutant character is teratological and leads to sterility. Except one case new genes

are recessive. Four genes are ever-mutable. Each new recessive gene may be considered to have taken place in the diploid cell, as shown by the results of some following generations, i.e. AA→Aa. It is quite the same in respect to the new origin of ever-mutable genes and also to that of somatic dominant mutations (= aa→aA).

370. Über den Wechsel der Baumtemperatur. (Japanisch). Kogo TOGASHI. (Agric. & Hortic. **56**, 1931, 531–546, m. 11 graphischen Darstellungen).

Inbezug auf einem 24 cm dicken Ast eines Pfirsichbaumes hat der Verf. an seiner Nord- und Südseite je einen ungefähr 10 cm breiten Loch gemacht, welcher 10 cm tief ist und somit ungefähr einen Drittel des ganzen Stammdurchmessers ausmacht. Mittelst des RICHARDschen " thermomètre enregisteur " hat er dabei eine langdauernde Messung des im Bauminnern herrschenden Temperaturwechsels gemessen, um ihn mit demselben der Umgebung zu vergleichen. Einige Resultate der Messung sind wie folgt. Bei dem nicht unter dem direkten Sonnenlichte wachsenden Baum stimmt der Temperaturwechsel im Bauminnern mit demselben der äussern Luft ziemlich gut überein. Nach dem Blattfall wird der Unterschied der Temperatur zwischen beiden bemerkbar. Auch der Temperaturunterschied an der Nord- und Südseite des Baumes wird beträchtlich, so z.B. in einem Fall konnte der Verf. beobachten, dass die Südseite um 20°C und die Nordseite um 10°C höher ist als die äussere Luft. Beim Regen oder Schnee ist der Temperaturwechsel im Bauminnern nur gering.

Inbezug auf die anderen Einzelheiten sei auf das Original verwiesen.

371. A New Species of *Blastospora*. Kogo TOGASHI and Fusaji ONUMA. (Bot. Mag., Tôkyô **45**, 1931, 4–7, with figs.).

A new species, *Blastospora Itoana* on leaves of *Smilax Oldhami* is described.

372. Über die Samenbildung bei den *Lycoris*arten. (Japanisch.) Yoshichika TOKUGAWA und Yoshikadzu EMOTO. (Bot. Mag. Tôkyô **44**, 1930, 236–244, 8 Textabb.).

In *Lycoris sanguinea* sind die Pollenkörner fast ausnahmslos gut ausgebildet und keimfähig, und in der freien Natur entstehen daraus gute keimfähige Samen. In *L. albiflora*, wobei die Pollenkörner grösstenteils schlecht ausgebildet sind, findet keine Samenbildung statt in der Natur, ebensowenig wenn der aus der Mutterpflanze ausgetrennte Blütenschaft mit seiner Basis im Wasser gelegt wird. Weder bei *L. radiata* noch *squamigera* sieht man in der Natur die Samenbildung, doch wenn man ihren Blütenschaft mit seiner Basis im Wasser legt, entsteht eine kleine Anzahl von anscheinend gut ausgebildeten Samen, welche aber nicht selten den Embryo entbehren und immer sich keimungsunfähig erwiesen haben. (Vgl. Nr. 316).

373. Über die Entwicklung des nackten Embryos von *Crinum latifolium* L. Kôgorô TOMITA (Sc. Rpts. Tôhôku Imp. Univ. Ser. IV, **6**, 1931, 163–169, 5 Textfig.).

Die Embryosackentwicklung von *Crinum latifolium* erfolgt normalerweise nach dem *Scilla*typ, wobei die haploide Zahl der Chromosome um 12 bestimmt wurde.

Inbezug anf den nackten Embryo, welcher zuerst von NAKAJIMA untersucht wurde (vgl. Japan. Jour. Bot. **4**, (44), Nr. 121) hat der Verf. festgestellt, dass er aus einer befruchteten Eizelle ankommt, und zwar ohne von der Endospermbildung begleitet zu werden, indem der sekundäre Embryosackkern ungeteilt bleibt. NAKAJIMA hat auch experimentell nachgewiesen, dass bei kastrierten Blüten der nackte Embryo nie entsteht, was seine parthenogenetische Entwicklung in Abrede stellt.

374. On the Recurring Mutation of Pine-needle Type of Morning Glory. (Japanese). Nagaharu U. (Japan. Jour. Gen., **6**, 1930, 199–202).

The so-called pine-needle type of Morning Glory is characterized by possessing slender leaves recalling pine needle as well as small flowers with 5-cleft corolla. Its two individuals have arisen in 1920 by mutation as the offspring of self-fertilized plant belonging to the pure race "Tonbobamaruzaki." It is completely sterile and could be preserved simply as a heterozygote of the latter. The pine-needle type is ever-sporting. This is due, according to the author, to an ever-mutable gene quite analogous to what TERAO has observed in the so-called large-grained race of rice (cf. Japan. Jour. Bot. **1**, (47), No. 117). He calculates the rate of transformation $x=0.0008$ and the coefficient $y=0.0328$. Like large-grained rice the chimeral phenomenon is often seen also, which consists in the production of the parts of the original parent in certain regions of the mutant. There are several kinds of such chimera which are due to the time difference of the factor transformation.

375. Formation of Haploid Plants in Morning Glory. (Japanese). Nagaharu U. (Japan. Jour. Gen. **6**, 1931, 203–204).

As the result of a certain cross experiment the author got among 422 offspring 1 aberrant plant which is tender, yellow-leaved and white-flowered. This plant is highly sterile. Pollen is of unequal size, and meiosis is very irregular. The chromosomes are irregularly scattered on the spindle in the metaphase. In anaphase 15 chromosomes are distributed mostly into 7 and 8, but sometimes 5 and 10. Laggards are often seen. The above plants are considered as haploids which possess the properties common to those hitherto known. The cause of the formation of such haploids may be due probaby to the fact that the egg nucleus was not properly fertilized and pseudogamy has taken place.

376. Heredity of Double Flowers in *Petunia hybrida*, hort. (Japanese). Nagaharu U. (Japan. Jour. Gen. **6**, 1931, 205).

The cross simple × double-flowered race of *Petunia hybrida* has given them in the 1:1 ratio. The cross double × double has given simple : double=1:3. That there are two kinds of double-flowered race is clear from the fact that some hybridization, double × simple produced the two in 1:1 ratio, while some other double × simple gave them in 2:1 ratio.

377. Notes on the Japanese Algae II. Yukio YAMADA. (Jour. Fac. Sc., Hokkaido Imp. Univ. Ser. V, **1**, 1931, 65–76, 5 pls.).

The following new species are described: *Cladophora japonica, Padina crassa, P. japonica, P. crispifolium, Besa gracilis, Rhodopeltis borealis.*

378. On the Morphology and Physiology of *Fomes applanatus* (FR.) GILL. and its Allies. Yoshiwo YAMANO. (Sc. Rpts. Tôhoku Imp. Univ. Ser. IV, **6**, 1931, 199-236, 4 pls. and 1 text-fig.).

Fomes vegetus which is closely allied to *F. applanatus* is treated by some biologists as a species quite distinct from the latter, and by others as its mere synonym. The author has studied 30 specimens of *Fomes* taken on a number of hosts of various kinds. He could distinguish three types which are externally quite similar, but distinguishable by their differences in the arrangement of the tube layers: in the first and the second types the context tissue or the white mycelial tissue is interposed between each annual tube layer respectivey, while in the third neither is seen there.

According to the results of the author's studies the three types show many conspicuous differences, neither in outer features of their sporophores nor in the spore characters (shape, structure of wall, size). Their behaviour in solid culture media have shown that in respect to their mode of growth and the temperature relation the second type is nearer to the first than to the third. In mixed culture no aversion phenomenon based on specific difference was recognized. In the culture on liquid media the effect of various nitrogen compounds and carbohydrates on the dry weight of fungi was studied, and it was found that the first and the second types are much more closely related to each other than to the third in this respect. On the basis of all facts above cited, both morphological as well as physiological, the author considers the second type as a variety of the first, viz. *F. applanatus* (FR.) GILL. var *leucostratus* and the third as a distinct species, viz. *F. vegetus* COOKE.

It may be added that the author was successful in extracting from these fungi several kinds of intracellular enzymes.

379. Testing of the Resistance of Varieties of Certain Plants to the Toxic Action of Potassium Chlorate with Seeds and Young Seedlings. (Japanese with English résumé). Morimasa YAMASAKI. (Jour. Imp. Agric. Exp. Sta. **1**, 1931, 267-304).

In various varieties of barley and wheat, the resistance against the toxic action of $KClO_3$ and that against cold are in negative correlation to each other. Just the same relation the author found in *Astragalus sinicus* and *Brassica campestris*. In rice the resistance against the toxic action of $KClO_3$ is much more intense in land varieties than in paddy ones, and of the former the more resistance against drought, the more resistant against the toxic action of $KClO_3$ also.

380. On the Cause of Varietal Distinctions in Certain Crop Plants in Regard to the Resistance to the Toxic Action of Potassium Chlorate. (Japanese with English résumé). Morimasa YAMASAKI. (Jour. Imp. Agric. Ext. Sta. **1**, 1931, 305-326, 6 pls.).

The toxic action of various salts which include potassium and sodium chlorate, potassium iodate and bromate, potassium and sodium perchlorate, sodium hyperchlorate, etc. on cereals and leguminous plants were studied. All salts used in the experiments are equally injurious, but in respect to the toxic action of potassium and sodium chlorate the author could observe wide variation in the susceptibility and resistance according to different varieties of plants taken. He thinks that the varietal difference of the toxic action of these two salts may be due to the ion ClO_3,

and not to any other ingredient. The symptom of poisoning by chlorate is quite identical to that by the hypochlorite NaClO, which is a well known strong poison. From the results of numerous experiments the author comes to the conclusion that the toxic action of chlorate is primarily due to the reduction of ClO_3 to ClO by some agency, such as glucose, aldehydes, etc. contained in plant cells. The varietal difference above mentioned is proportional to the quantity of the reducing matter contained in different varieties which effects the formation of ClO from ClO_3, i.e. the higher its content in plant cells, the greater their susceptibility toward the toxic action.

381. Über die Korrelationsverhältnisse bei den Weizen aus dem Standpunkte der Züchtung. (Japanisch). Morimasa YAMASAKI und Susumu HATANO. (Japan. Jour. Gen. **6**, 1930, 143–144).

Die Studien der F_2-Generation, welche von der Bastardierung zwischen langhalmig-spätschüssenden und kurzhalmig-frühschüssenden Weizensippen herrührt, zeigen, dass zwischen der Halmlänge und dem Schüssen der Ähre eine klare positive Korrelation besteht (Korrelationsindex = 49%, 68%). Die Bastardierung zwischen den langhalmig-behaarten and den kurzhalmig-unbehaarten Weizensippen hat in F_2 behaart: unbehaart = 3:1 ergeben, und dabei wurde es festgestellt, dass die Halme der behaarten im Mittel 4 cm länger sind als dieselben der unbehaarten.

382. An Experiment to Graft the Style upon the Ovary in *Petunia violacea*. Sadao YASUDA. (Proc. Imp. Acad. **7**, 1931, 672–675, 1 text-fig.).

In order to elucidate the fact, whether in self-sterile race of *Petunia violacea* the "Linienstoffe" (CORRENS) which inhibit the growth of its own pollen-tube in its style are secreted by the style itself or the ovary, the following experiments were carried out.

In the flowers of the A and B race the styles were cut off, and grafted by means of gelatine upon the ovaries of B and A flowers respectively with success. As the control the A and B styles were also grafted upon their own ovaries respectively. These grafted structures were then pollinated with the pollen of both A and B flowers, and it was ascertained that A pollen is effective when the ovary belongs to B, no matter whether the style is derived from A or B. It is the same with B pollen, because it is effective when the ovary belongs to A, no matter whether the style is derived from A or B. In other words, the inhibition occurs when the ovary belongs to the same race as pollen. From these experiments the author comes to the conclusion that the "Linienstoffe" of CORRENS are secreted by the ovary, and not by the style.

383. A Metaxenia-like Phenomenon Found in Some Plants of Solanaceae. (Japanese with English résumé). Sadao YASUDA and Toshio KITAMURA. (Japan. Jour. Gen. **6**, 1931, 137–142).

When *Solanum citrullifolium* is pollinated with *S. Delilei* the fruits are larger than those of the mother plant, and when mature, they become dark-coloured, which is intermediate between the two parents. Again when *S. citrullifolium* is pollinated with *S. agregatum* the fruits are smaller than in either parent, and when mature, they assume the colour lying between that of the two parents. In respect to the

cause of the metaxenia-like phenomenon above mentioned the authors are inclined to the view of SWINGLE, though they point out some difficulties in explaining their own observations according to it.

384. On the Spermatogenesis in *Makinoa crispata* (ST.) MIYAKE. Hiroshi YAZAWA. (Cytologia **2**, 1931, 157–173, 36 text-figs.)

The author has studied the development of spermatozoids in *Makinoa crispata*. No centrosomes (or blepharoplasts) could be found at the spindle-poles in the divisions of the spermatid mother-cells. It is probable that they arise *de novo* in the cytoplasm in each spermatid as a tiny granule. This fact is quite contrary to what the author has observed in *Marchantia polymorpha*, where the centrosome was discernible at each spindle-pole in the division of the spermatid mother-cell, and also most likely in the penultimate division. In *Makinoa* the centrosome or blepharoplast, soon after its appearance, elongates and fragments into a certain number of small segments. At the same time the nucleus elongates also in the same direction as the blepharoplast.

385. Factors influencing the Perithecial Formation of *Aspergillus glaucus* LINK. (With Japanese résumé). Seiiti YONEMOTO and Huzio KATÔ. (Bull. Miyazaki Coll. Agric. and For. No. **3**, 1931, 59–94).

Experiments were done to elucidate the influence of various external factors on the perithecial formation of *Aspergillus glaucus*. Among them the temperature is very efficacious, 28°C being the optimum and 15°C the minimum. Neither light (incl. ultraviolet or red rays) nor the difference of water quantity has any conspicuous influence. In respect to chemical substances, carbohydrates, such as dextrose, glucose, maltose, laevulose, galactose, etc. accelerate the perithecial formation, while lactose prevents it. The nitrogenous substances are much less efficacious than carbohydrates, though peptone, ammonium citrate, asparagin are more or less efficacious.

The formation of perithecia is more or less prevented by the presence of other fungi.

386. Untersuchungen über die osmotischen Werte bei den Pflanzen auf dem Berge Hakkôda. Yoshiji YOSHII und Tadao JIMBO. (Sc. Rpts. Tôhoku Imp. Univ. Ser. IV, **6**, 1931, 259–283).

Der Verf. haben den osmotischen Wert einer grossen Anzahl von Gebirgspflanzen gemessen, so z.B.

Hochgebirgspflanzen, einschliesslich diejenigen, welche auf starkaustrocknenden Felstrümmergeröll wachsen . .	0,4 Mol.
xeromorphe Pflanzen auf der Strauchstufe des Berges Hakkôda	0,8 ,,
Schattenpflanzen im stark schattenden *Sasa*-Gebüsche . .	0,3 ,,

usw. usw.

Woraus man sehen kann, dass der osmotische Wert der Hochgebirgspflanzen sehr niedrig ist und die starke Saugkraft ihrer Saugzellen schwer verständlich macht, da ohne solche Kraft sie auf sonnigem Gebiet, wo starke Transpiration herrscht, gegen starke Trockenheit nicht erhalten könnten.

Die osmotischen Werte aller untersuchten Pflanzen–143 Arten im ganzen – welche in oekologischer Hinsicht verschieden sind, sind am Ende der Abhandlung tabellarisch angegeben.

387 Über die Schwimminsel im Hikatasee. (Japanisch). Sannosuke YUKINOURA. (Mitteil. aus der Schulfreundgesell. zu Morioka **55**, 1931, 31 S. und 2 Taf.).

Bei einer Bergregion, welche zwei Städte Akita und Morioka in Nordjapan verbindet, findet sich ein vom Walde umgebener Becken, welcher mehr als 900 m über das Meeresniveau gelagert ist. Ein in diesem Becken befindlicher See—Hikatanuma—beträgt im Umkreis 300 m. In seiner Mitte sieht man eine Schwimminsel, welche aus Torf und lebenden Pflanzen zusammengesetzt und höchstwahrscheinlich erst durch das Abtrennen eines Torfbodenteiles am Seebord entstanden ist. Der Verf. hat in dieser Insel 18 Pflanzenarten gefunden. Alle in dieser Insel sowie in ihrer Nähe gesammelten Pflanzen werden enumeriert.

388. Nucleoli of the Root Tip and Cambium. Conway ZIRKLE. (Cytologia **2**, 1931, 85–105, 4 pls. and 1 text-fig.).

The author, by the application of several kinds of fixing fluids which preserve plastin, chromatin and mitochondria in various combinations, has investigated the nucleoli in the root tips and cambium of *Pinus Strobus*. In this species of *Pinus*, as in many other gymnosperms, each nucleus contains many nucleoli, in average six. That each nucleolus contains two distinct substances, as often reported in other plants, is clearly seen in living resting nuclei, for they being differently refractory, the nucleolus appears vacuolate. In the resting cell it is attached to the thread of chromatic reticulum, and on the initiation of karyokinesis it becomes attached to the spireme. The plastin passes then to the spireme, and is distributed to the daughter cells to form a part of the chromosome. When the daughter cells are reorganized the plastin is again consolidated into the typical nucleoli of the resting cell. In angiosperms it was often seen that in nuclear division fragments of nucleolar material pass from the equatorial plane to the spindle-poles, but in *Pinus* no such phenomenon was observed.